(19) Eur päisch s Patentamt
European Pat nt Office
Office uropéen des brevets

(11) Publication number: **0 395 853 A1**

(12) EUROPEAN PATENT APPLICATION

(21) Application number: 90104246.5

(22) Date of filing: 06.03.90

(51) Int. Cl.5: C12N 15/12, C12P 21/08, C12P 21/02

The title of the invention has been amended (Guidelines for Examination in the EPO, A-III, 7.3).

(30) Priority: 07.03.89 EP 89104023
29.05.89 EP 89109656
20.07.89 EP 89113310

(43) Date of publication of application:
07.11.90 Bulletin 90/45

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

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(54) Recombinant interleukin-2 receptor.

(57) Recombinant IL-21Rβ chain or portions thereof, cDNA coding therefore, vectors containing said cDNA, hosts transfected by said vectors, and monoclonal antibodies to said recombinant IL-2Rβ or portions thereof.

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Recombinant Protein Receptor

This invention relates to receptors for interleukin-2, more particularly to the β-chain of the receptor, and to cDNA coding for the β-chain or parts thereof, vectors containing cDNA inserts coding for the β-chain, hosts transformed by such vectors and the cultivation of such hosts to produce the said β-chain.

Ample evidence has been accumulated that cytokines, a class of soluble mediators involved in cell-to-cell "communications", are essential in the regulation of the immune system. It has been known that cytokines induce proliferation, differentiation and activation of target cells through interaction with specific cell surface receptor(s). Interleukin-2 (IL-2), previously defined as T cell growth factor (1), is one of the best characterized cytokines, known to play a pivotal role in the antigen-specific clonal proliferation of T lymphocytes (T cells) (2). IL-2 also appears to act on other cells of the immune system such as immature thymocytes (3), B lymphocytes (B cells) (4), macrophages (5), natural killer cells (NK cells) (6), and lymphokine-activated killer cells (LAK cells) (7). These multifunctional properties of IL-2 have now opened up possibilities in the formulation of immunotherapies such as adoptive immunotherapy (8). More recently, IL-2 has been shown to function also on neural cells such as oligodendrocytes (9), suggesting a possible involvement of this cytokine in the central nervous system. Despite extensive studies on the IL-2 system in the context of basic and clinical immunology, information has been limited on the molecular mechanism(s) underlying the IL-2-mediated signal transduction (10).

The IL-2 receptor (IL 2R) is known to be unique in that it is present in three forms: high-, intermediate- and low-affinity forms with respect to its binding ability to IL-2, and respective dissociation constants (Kds) of 10^{-11}M, 10^{-9}M and 10^{-8}M (11, 12). Following the characterization of IL-2Rα chain (Tac antigen, p55) (13), it became evident that the α chain constitutes solely the low-affinity form and it is not functional per se in IL-2 internalization and signal transduction, unless associated with another specific membrane component(s) of lymphoid cells (14, 15). Subsequently, the lymphoid membrane component was identified to be a novel receptor chain, termed β chain (or p70-75) (12, 16, 17). In fact, experimental evidence has suggested that the IL-2Rβ chain per se constitutes the intermediate-affinity form (12). In addition, its association with the IL-2Rα chain results in the high-affinity form of the receptor (12, 16, 17). Expression studies using wild type and mutated IL-2Rα chain cDNAs strongly support the notion that the IL-2Rβ chain but not the IL-2Rα chain possesses a domain(s) responsible for driving the intracellular signal transduction pathway(s) (18). There exists, therefore, a need to obtain IL-2β chain in amounts which will enable its structure and function to be elucidated, this being an essential step in gaining further insight into the molecular basis of the high-affinity IL-2R as well as on the mechanism of signal transduction operating in IL-2 responsive cells. To this end we describe below cDNA coding for the IL-2Rβ chain or parts thereof whereby insertion of said cDNA in a suitable vector and expression thereof in an appropriate host will enable recombinant and large scale production of protein corresponding to the IL-2Rβ chain or parts thereof.

According to a one aspect of the present invention therefore we provide a recombinant cDNA coding for an IL-2Rβ chain or a portion thereof.

cDNA of the invention may have the formula:

ATG
GCG GCC CCT GCT CTG TCC TGG CGT CTG CCC CTC CTC ATC
CTC CTC CTG CCC CTG GCT ACC TCT TGG GCA TCT GCA GCG
GTG AAT GGC ACT TCC CAG TTC AGA TGC TTC TAC AAC TCG
AGA GCC AAC ATC TCC TGT CTC TGG AGC CAA GAT GGG GCT
CTG CAG GAC ACT TCC TGC CAA GTC CAT GCC TGG CCG GAC
AGA CGG CGG TGG AAC CAA ACC TGT GAG CTG CTC CCC GTG
AGT CAA GCA TCC TGG GCC TGC AAC CTG ATC CTC GGA GCC
CCA GAT TCT CAG AAA CTG ACC ACA GTT GAC ATC GTC ACC
CTG AGG GTG CTG TGC CGT GAG GGG GTG CGA TGG AGG GTG
ATG GCC ATC CAG GAC TTC AAG CCC TTT GAG AAC CTT CGC
CTG ATG GCC CCC ATC TCC CTC CAA GTT GTC CAC GTG GAG
ACC CAC AGA TGG AAC ATA AGC TGG GAA ATC TCC CAA GCC
TCC CAC TAC TTT GAA AGA CAC CTG GAG TTC GAG GCC CGG
ACG CTG TCC CCA GGC CAC ACC TGG GAG GAG GCC CCC CTG
CTG ACT CTC AAG CAG AAG CAG GAA TGG ATC TGC CTG GAG
ACG CTC ACC CCA GAC ACC CAG TAT GAG TTT CAG GTG CGG
GTC AAG CCT CTG CAA GGC GAG TTC ACG ACC TGG AGC CCC
TGG AGC CAG CCC CTG GCC TTC AGG ACA AAG CCT GCA GCC
CTT GGG AAG GAC ACC ATT CCG TGG CTC GGC CAC CTC CTC
GTG GGC CTC AGC GGG GCT TTT GGC TTC ATC ATC TTA GTG
TAC TTG CTG ATC AAC TGC AGG AAC ACC GGG CCA TGG CTG
AAG AAG CTC CTG AAG TGT AAC ACC CCA GAC CCC TCG AAG
TTC TTT TCC CAG CTG AGC TCA GAG CAT GGA GGA GAC GTC
CAG AAG TGG CTC TCT TCG CCC TTC CCC TGA TCG TCC TTC
AGC CCT GGC GGC CTG GCA CCT GAG ATC TCG CCA CTA GAA
GTG CTG GAG AGG GAC AAG GTG ACG CAG CTG CTC CTG CAG

CAG GAC AAG GTG CCT GAG CCC GCA TCC TTA AGC AGC AAC
CAC TCG CTG ACC AGC TGC TTC ACC AAC CAG GGT TAC TTC
TTC TTC CAC CTC CCG GAT GCC TTG GAG ATA GAG GCC TGC
CAG GTG TAC TTT ACT TAC GAC CCC TAC TCA GAG GAA GAC
CCT GAT GAG GGT GTG GCC GGG GCA CCC ACA GGG TCT TCC
CCC CAA CCC CTG CAG CCT CTG TCA GGG GAG GAC GAC GCC
TAC TGC ACC TTC CCC TCC AGG GAT GAC CTG CTG CTC TTC
TCC CCC AGT CTC CTC GGT GGC CCC AGC CCC CCA AGC ACT
GCC CCT GGG GGC AGT GGG GCC GGT GAA GAG AGG ATG CCC
CCT TCT TTG CAA GAA AGA GTC CCC AGA GAC TGG GAC CCC
CAG CCC CTG GGG CCT CCC ACC CCA GGA GTC CCA GAC CTG
GTG GAT TTT CAG CCA CCC CCT GAG CTG GTG CTG CGA GAG
GCT GGG GAG GAG GTC CCT GAC GCT GGC CCC AGG GAG GGA
GTC AGT TTC CCC TGG TCC AGG CCT CCT GGG CAG GGG GAG
TTC AGG GCC CTT AAT GCT CGC CTG CCC CTG AAC ACT GAT
GCC TAC TTG TCC CTC CAA GAA CTC CAG GGT CAG GAC CCA
ATC CAC TTG GTG TAG

which codes for human Il-2Rβ, or a degenerate variant thereof or a portion thereof.

Another cDNA of the invention for instance has the formula:

ATG

GCT ACC ATA GCT CTT CCC TGG AGC CTG TCC CTC TAC GTC TTC CTC CTG CTC CTG GCT ACA CCT TGG GCA TCT GCA

GCA GTG AAA AAC TGT TCC CAT CTT GAA TGC TTC TAC AAC TCA AGA GCC AAT GTC TCT TGC ATG TGG AGC CAT CAA

GAG GCT CTG AAT GTC ACA ACC TGC CAC GTC CAT GCC AAG TCG AAC CTG CGA CAC TGG AAC AAA ACC TGT GAG CTA

ACT CTT GTG AGG CAG GCA TCC TGG GCC TGC AAC CTG ATC CTC GGG TCG TTC CCA GAG TCC CAG TCA CTG ACC TCC

CTG GAC CTC CTT GAC ATA AAT GTG GTG TGC TGG GAA GAG AAG GGT TGG CCT AGG CTA AAG ACC TGC GAC TTC CAT

CCC TTT GAC AAC CTT CGC CTG GTG GCC CCT CAT TCC CTC CAA GTT CTG CAC ATT GAT ACC CAG AGA TGT AAC ATA

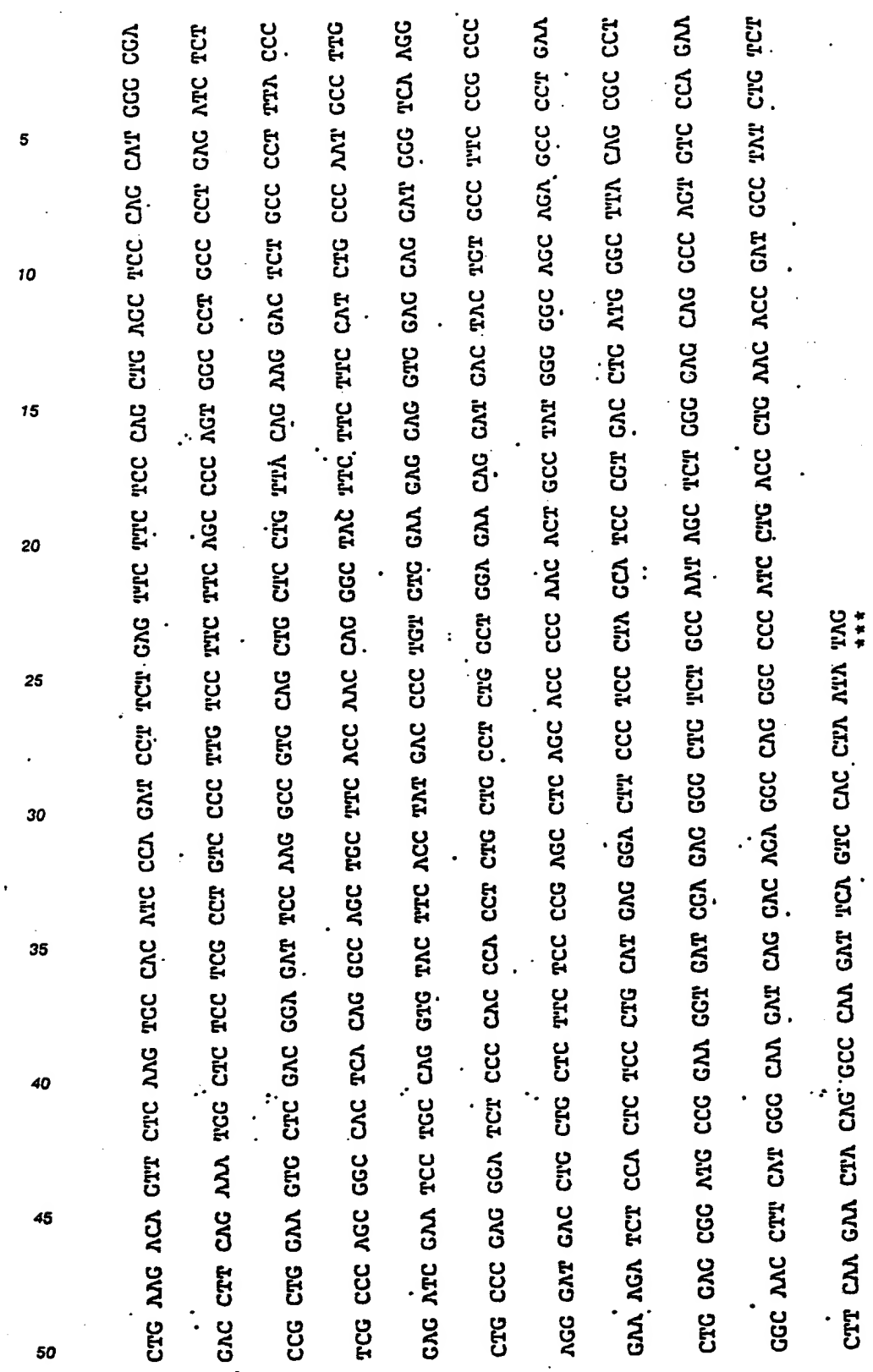
AGC TGG AAG GTC TCC CAG GTC TCT CAC TAC ATT GAA CCA TAC TTG GAA TTT GAG GCC CGT AGA CGT CTT CTG GGC

CAC AGC TGG GAG CAT GCA TCC GTA TTA AGC CTC AAG CAG AGA CAG ACG TGG CTC TTC TTG GAG ATG CTG ATC CCT

AGT ACC TCA TAT GAG GTC CAG GTG AGG GTC AAA GCT CAA CCA AAC AAT ACC GGG ACC TGG AGT CCC TGG AGC CAG

CCC CTG ACC TTT CGG ACA AGG CCA GCA GAT CCC ATG AAG GAG ATC CTC CCC ATG TCA TGG CTC AGA TAC CTT CTG

CTG GTC CTT GGT TGT TTT TCT GGC TTC TTC TCC TGC GTC TAC ATT TTG GTC AAC XXX CGC TAC CTT GGG CCA TGG



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CTG AAG ACA GTT CTC AAG TGC CAC ATC CCA GAT CCT TCT GAG TTC TTC TCC CAG CTG AGC TCC CAG CAT GGC CGA
GAC CTT CAG AAA TGG CTC TCC TCG CCT GTC CCC TTG TCC TTC TTC AGC CCC AGT GGC CCT GCC CCT CAG ATC TCT
CCG CTG GAA GTG CTC GAC GGA GAT TCC AAG GCC GTG CAG CTG CTC CTG TTA CAG AAG GAC TCT GCC CCT TTA CCC
TCG CCC AGC GGC CAC TCA CAG GCC AGC TGC TTC ACC AAC CAG GGC TAC TTC TTC CAT CTG CCC AAT GCC TTG
CAG ATC GAA TCC TGC CAG GTG TAC TTC ACC TAT GAC CCC TGT GAA GAG CAG GTC GAC CAG CAT GGG TCA AGG
CTG CCC GAG GGA TCT CCC CAC CCA CCT CTG CTC CCT CTG GCT GGA GAA CAG CAT CAC TAC TGT GCC TTC CCG CCC
AGG GAT GAC CTG CTG CTC TTC TCC CCG AGC CTC AGC ACC CCC AAC ACT GCC TAT GGG GGC AGC AGA GCC CCT GAA
GAA AGA TCT CCA CTC TCC CTG CAT GAG GGA CTT CCC TCC CTA GCA TCC CGT GAC CTC ATG GGC TTA CAG CGC CCT
CTG GAC CGC ATG CCG GAA GGT GAT CGA GAC GGC CTG TCT GCC AAT AGC TCT GGC CAG CCC AGT GTC CCA GAA
GGC AAC CTT CAT GGC CAA GAT CAG GAC AGA GGC CAG GGC CCC ATC CTG ACC CTG AAC ACC GAT GCC TAT TCT CTG TCT
CTT CAA GAA CTA CAG GCC CAA GAT TCA GTC CAC CTA ATA TAG
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XXX = CCC or TGC
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which codes for murine IL-2Rβ, or a degenerat variant thereof or a portion thereof.

DNAs of paricular interest in the present invention include those coding for portions of the IL2-Rβ, for instance the extracellular part or a portion thereof or the intracellular part or a portion thereof.

Of especial interest are those DNAs coding for soluble parts of IL2-Rβ, these including the extracellular parts and portions thereof.

The pres nt invention thus also includes within its scope cDNA coding for portions of the above

mentioned cDNAs, e.g. portions of the complete sequence of the hIL-2Rβ chain, for instance the extracellular portion beginning at, or about amino acid (a;a) (see Fig. 1 B) 1 e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and ending at or about a.a. 214 e.g. 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 214, 215, 216, 217, 218, 219, 220, or sub-portions of this extracellular part or degenerate variants thereof, or portions corresponding to the intracellular part of the receptor chain e.g. the portion beginning at or about a.a. 239 e.g. a.a. 230, 231, 232, 234, 235, 236, 237, 238, 239, 240, 241, 242, up to or about the end a.a. 525, e.g. 516, 517, 518, 519, 520, 521, 522, 523, 524 and 525 or sub-portions ther of, or degenerate variants thereof, as well as cDNA coding for portions of the complete sequence of the murine IL-2Rβ chain (see Fig. 8) for instance the extracellular part beginning at, or about amino acid 1 e. g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and ending at or about amino acid 210 e.g. 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220 or sub-portions of this extracellular part or degenerate variants thereof, or portions corresponding to the intracellular part of the receptor chain e.g. the portion beginning at or about amino acid 235 e.g. amino acid 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, up to or about the end a.a. 513 e.g. 505, 506, 507, 508, 509, 510, 512, and 513 or sub-portions thereof or degenerate variants thereof.

It will be understood that for the particular IL-2Rβ chains or portions thereof described herein, natural allelic variations may exist, occuring from individual to individual. These variations may be demonstrated by one or more amino acid differences in the overall sequence or by deletions, substitutions, insertions, inversions or additions of one or more amino acids in the sequence. In addition it will be understood that the IL-2Rβ chain or portions thereof described herein may be modified by genetic engineering techniques e.g. point mutation, for the substitution, deletion or addition of one or more amino acids without changing the essential characteristics of the IL-2Rβ chain or portion thereof. The present invention thus also includes within its scope DNA sequences capable of hybridising with the DNA sequences described herein and coding for proteins having substantially the activity of an Il-2Rβ chain or portions thereof, especially soluble IL-2Rβ.

In one further aspect of the invention we provide a recombinant DNA molecule coding for a water soluble portion of the human IL2-Rβ (hIL2-Rβ) for example an amino acid sequence comprising the amino acids about 1 to about 210 of the entire hIL2-Rβ. Such a DNA molecule may code, for example, for a soluble human interleukin 2 receptor β-chain derivative having 212 amino acid residues in which residues 1 to 210 correspond to the amino acids of the native IL-2Rβ-chain.

For example in one embodiment described below the terminal nucleotides of a cDNA molecule coding for a soluble hIL-2Rβ derivative are as follows

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GCC CTT GCT AGC TAG
208 Ala Leu Ala Ser [Stop]
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Using standard techniques of recombinant DNA technology vectors for transforming suitable host cells can be constructed which contain cDNA sequences corrsponding to the structural gene for IL-2Rβ as set forth above or any desired portion thereof, or degenerate variant thereof.

Suitable vectors are plasmid vectors for example and will include control and regulatory sequences operably linked to the cDNA sequence coding for the IL-2Rβ chain or portion thereof.

Suitable techniques are well known and widely practised and by way of Example are described, in connection with other proteins, in European Patent Applications, Publication Nos. 0254249 and 0170204.

Obtaining the desired protein in pure form from the culture can be carried out by standard techniques and such protein provides a suitable antigen for preparing monoclonal antibodies. Thus hybridomas capable of secreting a monoclonal antibody having a specific affinity to the IL-2Rβ chain or a desired portion thereof may be prepared by immunizing a non-human animal with recombinant IL-2Rβ or a portion thereof, removing spleen cells with non-immunoglobulin secreting myeloma cells, and selecting from the resulting hybridomas a cell line which produces a monoclonal antibody having the desired binding specificity and, if desired, subsequently sub-cloning said hybridoma.

The techniqu s for preparing hybridomas and obtaining monoclonal antibodies in pure form therefrom are well known and by way of example are described in European Patent Application, Publication No. 0168745.

Antibodies in accordance with the invention are useful .g. for diagnostic purposes and also for therapy by immun suppression or activation. As mentioned above, such antibodies could be raised using purified recombinant protein in accordance with the invention or by transfecting the cDNA of the invention, obtaining

cells expressing large amounts of the receptor and using such cells to obtain the antibodies.

As indicated above the present invention envisages soluble forms of IL-2Rβ chain and of soluble IL-2 receptor. Soluble forms include those coded for by the partial cDNA sequences coding for the extracellular part of IL-2Rβ or subportions thereof as described above. If desired both IL-2Rβ chain and α-chain may be produced simultaneously.

The availability of monoclonal antibodies to specific sub-portions of the IL-2β chain enables epitopes of the receptor chain to be identified and thus opens the way for control of the activity of the receptor to be excercised using suitable monoclonal antibodies or other peptides or peptidomimetic or protein analogue substances.

Isolation and analysis of the cDNA clones coding for human IL-2Rβ chain

In isolating the cDNA clones, an expression cloning strategy was applied by using the monoclonal antibodies, Mik-β1 and Mik-β2 (19), both of which have been raised against the IL-2Rβ chain found on the human leukemic cell line YT (20).

The monoclonal antibodies Mik-β1 and Mik-β2 are both deposited at Fermentation Research Institute, Agency of Industrial Science and Technology, Japan. The deposit numbers for Mik-β1 and Mik-β2 are, 10453 and 10454 (1988), respectively; they are also described in Japanese Patent Application No. 298742 (1988).

A few sets of cDNA libraries were prepared by using the poly(A)$^+$-RNA from YT cells according to standard procedures. cDNA libraries were prepared with cDM8 vector according to published procedures (21), except using random primer (Amersham) or oligo (dT) primer as mentioned below. The plasmid DNA representing 5.6×10^6 independent colonies was prepared by the standard procedure and one mg of DNA was used for the first DNA transfection. Actually, the DNA was divided into 100 tubes (therefore each tube contained 10 μg of DNA) and they were each transfected into 3.5×10^5 monkey COS cells in a tissue culture dish (60 mm polystyrene dish, Corning). The transfection was done using the standard DEAE dextran procedures. The transfected COS cells were then treated with the cocktail of Mik-β1 and -β2 antibodies (400-fold diluted ascites for each antibody) and subjected to the standard panning procedure. The dish used for the panning was FALCON 60 mm dish, coated with anti-mouse IgG as described previously (ref. 21). In this first round of panning, 100 IgG-coated dishes were used. After the panning, Hirt extract was prepared by the standard procedure (ref. 21) and the recovered plasmids were introduced into E.coli by the method described in ref. 21. By this procedure 3.7×10^6 colonies were obtained. Those bacterial colonies were fused with COS cells by the standard protoplast fusion procedures (ref. 21). In these fusion experiments, 26 Corning dishes each containing 5×10^5 COS cells were used. After the fusion, the COS cells were subjected to panning as described above and Hirt extract was prepared. 32,000 bacterial colonies were obtained from the Hirt extract. The fusion, panning procedures were repeated again and 32,000 bacterial colonies were obtained from the subsequent Hirt extract. The same procedures were repeated once again, obtaining 28,000 bacterial colonies (in the meantime, there should be a dramatic enrichment of the objective clones). The same procedures were repeated once again and 6,000 colonies were obtained. From these colonies, 30 colonies were picked up randomly and the cDNA inserts were analysed. Of them, only 7 colonies contained plasmids from which cDNA inserts can be excised by restriction enzyme XhoI. The vector derived XhoI sites were located at both sides of the cDNA and all other plasmids had lost such cleavage sites due to the DNA rearrangements; in fact, all of them were much smaller in size than the original vector. Thus they were considered to be non-specific products. On the other hand, all of the 7 colonies were derived from the same mRNA, as confirmed by the conventional restriction enzyme cleavage analysis and DNA blot analysis. Of them, one plasmid, termed pIL-2Rβ30 contained longer cDNA than the other 6 plasmids which turned out to be identical to each other (designated pIL-2Rβ9)

In this procedure, therefore, we isolated two independent cDNA clones, pIL-2Rβ9 and pIL-2β30; each of the expression products specifically reacted with the antibodies. The two clones contained cDNA inserts of 1.3Kb and 2.3Kb, respectively, and cross-hybridized with each other. Subsequent sequence analysis of the cDNAs revealed that they represent the same mRNA. In fact, RNA blotting analysis revealed that the mRNA is approximately 4Kb in size (see below). Subsequently, we screened other YT cDNA libraries by using the clone d cDNAs as probes, and several independent cDNA clones which together cover the entire mRNA for the IL-2Rβ chain were isolated. Thus pIL-2Rβ6 and pIL-2Rβ19 were obtained by screening the cDNA libraries with the pIL-2Rβ9 cDNA insert in the probe.

The above mentioned plasmids containing cDNA coding for human IL-2Rβ sequences have been deposited in strain E.coli MC 1061/P3 on March 2, 1989 at the Fermentation Research Institute according to

the Budapest Treaty under the following accession numbers:

Plasmid	Accession No.
pIL-2Rβ6	FERM BP-2312
pIL-2Rβ9	FERM BP-2313
pIL-2Rβ19	FERM BP-2314
pIL-2Rβ30	FERM BP-2315

The complete nucleotide sequences of four of the cloned cDNAs were determined (Fig. 1).

Fig. 1 shows the structure of the human IL-2Rβ chain cDNA. Fig. 1a is a schematic representation of the mRNA as deduced from the cloned cDNAs. Dotted, hatched, open and closed rectangles representing respectively the signal sequence, the extracellular, the transmembrane and the cytoplasmic regions of the mRNA are shown below. Fig. 1b shows the nucleotide and amino acid sequences of the human IL-2Rβ chain cDNA. The sequence was deduced following the complete DNA sequence analysis of the above described cDNA clones. Nucleotides are numbered on the right margin and amino acids are numbered on the left margin. Clones pIL-2Rβ19 and pIL-2Rβ6 each contained G-A mutation at nucleotide residues 425 and 1531, respectively. Thus pIL-2Rβ6 cDNA acquired a TAG triplet in the cytoplasmic region. It is thought to be an error in reverse transcription, since all other clones, pIL-2Rβ30, pIL-2Rβ19 and pIL-2Rβ16, have a TGG triplet at that position. The first underlined 26 amino acid residues represent the signal sequence as predicted by the consensus sequence (22). The 25 transmembrane amino acid residues are marked with a thick underlining. The cysteine residues are boxed. The potential N-glycosylation sites are underlined twice. The possible poly-adenylation signals are shown by open rectangle. In summary, RNA was prepared from the NK-like human lymphoid cell line, YT, and cDNA libraries were prepared with CDM8 vector according to published procedures (21), except using either random primers (Amersham) (for pIL-2Rβ6, 9 and 30), or oligo (dT) primer (for pIL-2Rβ19). Screening of the cDNA libraries by a cocktail of anti-IL-2Rβ monoclonal antibodies, Mik-β1 and Mik-β2, was carried out as described previously (21). Nucleotide sequences were determined by a combination of dideoxy chain termination and chemical cleavage methods.

As shown in Fig. 1, the cDNA contains a large open reading frame that encodes a protein consisting of 551 amino acids. No significant homology with other known proteins was found in the protein Sequence Database (National Biomedical Research Foundation, Washington, D.C.) or with sequences published more recently. Unlike many of other cytokine receptors, it appears that IL-2Rα and Il-2Rβ chains do not belong to the immunoglobulin superfamily. From the deduced structure of the protein, the N-terminal 26 amino acids is considered to represent the signal sequence. Thus the mature form of the IL-2Rβ chain consists of 525 amino acids with a calculated M.W. of 58.358. As shown in Fig. 1, the receptor molecule consists of an extracellular region consisting of 214 amino acids. This region contains 8 cysteine residues of which 5 residues are found in the N-terminal half and they are interspaced rather periodically by 9-12 amino acids. It is likely that disulfide linkages between the cysteine residues impart a stable configuration for ligand binding. In fact, abundance of cysteine residues seems to be one of the common features of the ligand binding domain of many receptors (23). It may be worth noting that the predicted number of amino acids (a.a.) within the extracellular region of the Il-2Rβ chain (214 a.a.) is almost comparable in number to that of the IL-2Rα chain (219 a.a.). Such size similarity may be significant in considering the conformation of the heterodimeric receptor complex that is quite unique for this receptor; as both α and β chains individually interact with distinct sites of the same IL-2 molecule (24).

A hydrophobic stretch of 25 amino acids spanning from the 215 to 239 amino acid residues appears to constitute the membrane spanning region of the receptor (Fig. 1 and 2).

Fig. 2 is a hydropathy plot analysis of deduced human IL-2Rα and Il-2Rβ chain precursor structures. The analysis was carried out according to Kyte and Doolittle (38). SG and TM represent signal sequence and transmembrane sequence respectively.

The cytoplasmic region of the β chain consists of 286 a.a. and it is far larger than that of the α chain, which is only 13 a.a. long. The consensus sequences of tyrosine kinase (Gly-x-Gly-x-x-Gly) (25) are absent in the β chain. However, th pr sence of a triplet, Ala-Pro-Glu (293-295) may be noted; this has b en asserted to be the consensus motif for a catalytic domain of some protein kinases (25). Th possibility of the cytoplasmic region of the β chain having a protein kinase activity has yet to be tested. The primary structure of this region revealed yet another interesting feature; a rather strong bias for certain characteristic amino acids. This region is rich in prolin (42/286) and serine (30/286) residues. Interestingly, the "proline rich" structure has also been demonstrated in the cytoplasmic region of CD2, a T cell membran antigen

involved in the activation pathway of T cells (26). The proline-rich structure may impart a non-globular conformation to this region that may be important in coupling of the receptor molecul with other signal transducer(s). The predominant serine residues may be the major target for phosphorylation, which could also modulat the receptor function (27). In addition, the cytoplasmic region is notably biased for negatively charged amino acids. In fact, this region contains 40 such amino acids (i.e. glutamic and aspartic acids), whereas only 18 amino acids account for the positively charged residues (i.e. lysine and arginine). Such a bias is particularly notable in the middle portion (a.a. 345-390) of the cytoplasmic region. Thus, the cytoplasmic region of the β chain may be quite acidic. Taken together some, if not all, of these unique characteristics may be responsible in driving further the downstream signal transduction pathway(s). The receptor protein contains 5 potential sites for N-linked glycosylation (Fig. 1B), of which 4 are located in the extracellular region. Such a posttranslational modification may account for the difference between the M.W. of the estimated mature (70-75kD) and the calculated (58kD) protein molecules. Hydropathy plot analysis of the α and β chains revealed the presence of hydrophilic regions just adjacent to the cell membrane in the both chains (Fig. 2) These regions may play a role in the non-covalent intramolecular association between the two chains.

Expression of Human IL-2Rβ chain mRNA

Expression of the IL-2Rβ mRNA was examined by using the cDNA insert from pIL-2Rβ30 as the probe

Fig. 3a illustrates the expression of human IL-2Rβ chain mRNA in different cell types. Poly(A)$^+$RNA (2μg per lane) from the following cell sources was prepared and subjected to RNA blotting analysis using the XhoI-digested human IL-2Rβ chain cDNA fragment derived from pIL-2Rβ30 as a probe following standard procedures (14, 18, 27). Lane 1, YT; lane 2, Hut102(HTLV-1 transformed human T cell line); lane 3, MT-2(HTLV-1 transformed human T cell line); lane 4, ARH-77 (multiple myeloma line); lane 5, SKW6.4 (EBV-transformed human B lymphoblastoid line); lane 6, U937 (histiocytic leukemia line); lane 7, MT-1 (HTLV-1 transformed human T cell line); lane 8, Jurkat (human T leukemic line); lane 9, HeLa (human cervical carcinoma cell line).

As shown in Figure 3a, the RNA blot analysis revealed the presence of a 4kb mRNA, the expression of which is restricted to lymphoid cells previously identified to bear IL-2Rβ chain (i.e. YT, MT-2, Hut102, SKW6.4) (12, 16, 17). On the other hand, the mRNA expression was not detected in cells such as Jurkat, MT-1, U937, ARH-77 and HeLa cells. Essentially, the mRNA expression levels are in correlation with the IL-2Rβ chain expression levels.

Fig. 3 b illustrates the expression of IL-2Rβ and IL-2Rα mRNAs in human PBLs. Total RNA (15μg per lane) was loaded in each lane. Lanes 1 and 4 represent unstimulated human peripheral blood lymphocytes (PBLs); lanes 2 and 5, PBLs stimulated with 5μg/ml phytohemagglutinin (PHA) for 24 hrs; lanes 3 and 6, PBLs stimulated with 5μg/ml PHA for 72 hrs. The RNA-blotted filter was hybridized with the IL-2Rβ probe (lanes 1-3). After dehybridization of the IL-2Rβ probe, the same filter was hybridized with the IL-2Rα probe (XbaI-BclI fragment derived from pSVIL2R-3 (14) (lanes 4-6).

Interestingly, the IL-2Rβ mRNA was detectable in the unstimulated PBLs and its expression levels increased transiently only 2.5-fold after mitogen stimulation. Based on previous data derived from flow cytometric analysis (19), it is likely that the mRNA induction patterns differ between the different lymphocyte populations. This expression pattern is quite different from that of the IL-2Rα chain whose expression strictly requires mitogenic stimulation of the cells (Fig. 3b), suggesting the presence of distinct mechanisms of gene expression between the two genes.

Southern blot analysis of the genomic DNA from PBL and various cell lines including HTLV-1-transformed human T cell lines indicates that the gene is present in a single copy and is not rearranged in those cells.

IL-2 binding properties of the cDNA-encoded IL-2Rβ chain

We next carried out a series of cDNA expression studies in order to examine whether the cDNA product binds IL-2 and indeed manifests the properties of the IL-2Rβ chain that have been demonstrated and/or suggested in previous studies. Two cDNA expression plasmids were constructed in which expression of the cDNA spanning the entire coding region was directed by either the mouse lck gene (29) promoter (pLCKRβ) or Moloney leukemia virus LTR (30) (pMLVRβ).

Expression vectors were constructed by the following procedures. pIL-2Rβ30 was digested with HindIII

(th cleavage sit is located within the polylinker r gions of CDM8) and, after fill-in of both ends, a BamHI linker was attached and religated. The resulting plasmid was then digested with BamHI and the 1.8kb DNA fragment which contains the ntire coding sequence for the β chain was introduced into BamHI-cleaved p1013 vector containing the mouse lck promot r to construct pLCKRβ. The BamHI-dig sted cDNA fragm nt was also introduced into a retrovirus vector, pZipSV(X) (30), to construct pMLVRβ. The human IL-2Rα expression vector, pSVIL2Rneo, was obtained from pSVIL2R-3 (14) by replacing th Eco-gypt gene with the neo-resistance gene.

The plasmid pLCKRβ was introduced into the mouse T lymphoma EL-4 and the human T cell leukemia Jurkat lines, both of which are known to be devoid of surface molecules that bind human IL-2.

Transfection of the expression plasmids into Jurkat and EL-4 cells was carried out by electroporation as described previously (39). Transfected cells were selected in the RPMI1640 medium containing 10% fetal calf serum (FCS) and G418 (1 mg/ml for EL-4 and 1.5 mg/ml for Jurkat). To obtain cells expressing cDNAs for human IL-2Rα and IL-2Rβ chains simultaneously, a Jurkat-derived clone Jα-5, transfected with pSVIL2Rneo, was co-transfected with pLCKRβ and a plasmid containing the hygromycin-resistance gene, pHgy. The transfected cells were selected with 200μg/ml hygromycin. Transfection of pMLVRβ into 2 cells was carried out by calcium-phosphate method as described previously (14) and the cells were selected by 700μg/ml of G418. For flow cytometric analysis, $5x10^5$ cells were treated with antibody (1:500 dilution of ascites) at 4° C for 30 min. After washing, cells were stained with fluorescein-conjugated goat anti-mouse IgG.

The stained cells were analysed on a FACS440 flow cytometer (Beckton Dickinson). The ^{125}I-IL-2 binding assay and Scatchard plot analysis were carried out as described previously (12).

Fig. 4a illustrates the expression of human IL-2Rα and/or IL-2Rβ chain cDNAs by means of cell surface staining patterns of human IL-2Rα and/or IL-2Rβ cDNA transfectants. Parental cells and various transfectant cells were separately stained with either a monoclonal anti-human IL-2Rα antibody, anti-Tac (——), or monoclonal anti-human IL-2Rβ antibody, Mik-β1 (————). Dotted line (.....) is a fluorescence profile of the cells stained with fluorescein-conjugated goat-anti-mouse IgG alone. Cells used were (1) ELβ-13 (and EL-4-derived clone transfected with pLCKRβ), (2) Jβ-8 (a Jurkat-derived clone transfected with pLCKRβ), (3) Jα-5 (a Jurkat-derived clone transfected with pSVIL2Rneo), (4) Jα-2 (a Jα-5-derived clone transfected with pLCKRβ), (5) Jαβ-10 (a Jα-5-derived clone transfected with pLCKRβ), and (6) Fβ-3 (a NIH3T3-derived line transfected with pMLVRβ).

Stable transformant clones expressing the cDNA product were obtained for both the EL-4 (ELβ-13) and Jurkat (Jβ-8) cells as judged by FACS analysis (Fig. 4a). In addition, we also introduced the same gene into the Jurkat transformant clone, Jα-5, which expresses the transfected, human IL-2Rα chain cDNA. Two of the resulting transformants, Jαβ-2 and Jαβ-10, were found to express both α and β chains (Fig. 4a-(4), (5)). As expected, RNA blotting analyses of the mRNA expressed in those transformants revealed that the α and β chain-specific mRNAs are derived from the transfected cDNAs but not from the endogeneous genes (26). Furthermore, in order to examine the property of the cDNA product in non-lymphoid cells, the plasmid pMLVRβ was introduced into an NIH3T3 cell-derived cell line 2 (30), and the resulting transformant expressing the cDNA, Fβ-3, was obtained (Fig. 4a-(5)).

The IL-2 binding studies were carried out with ^{125}I-labeled, recombinant human IL-2.

Fig. 4b illustrates the expression of the α and β chains by means of the Scatchard plot analysis of ^{125}I-IL-2 binding to the transfectants expressing the cloned cDNAs. Scatchard plot of the IL-2 binding data in the absence (-o-o-) or presence (-●-●-) of 1:100-diluted ascites of Mik-β1. Binding of ^{125}I-IL-2 to ELβ-13 or Jβ-8 was completely abolished by Mik-β1. No specific IL-2 binding was observed when parental Jurkat or EL-4 cells were examined. The number of IL-2 binding sites per cell and the receptor affinity were determined by computer-assisted analysis of the IL-2 binding data. (1) ELβ-13, (2) Jβ-8, (3) Jα-5, (4) Jαβ-2, (5) Jαβ-10.

As can be seen the EL-4-derived clone (ELβ-13) and the Jurkat-derived clone (Jβ-8), both expressing the β chain cDNA displayed intermediate-affinity to IL-2 with estimated Kd values of 4.0nM and 2.7nM, respectively. The IL-2 binding to those cells was completely abolished by the Mik-β1 antibody (Fig. 4b-(1), (2)). The Jurkat-derived Jαβ-2 and Jαβ-10 clones expressing both the human IL-2Rα and Il-2Rβ cDNA displayed both high and low affinity receptors with estimated Kp values of 22pM and 15nM for Jαβ-2 and 19pM and 33nM for Jαβ-10, respectively. In contrast, the parental, Jurkat-derived Jα-5 cells expressing the α chain cDNA alone manifested exclusively low-affinity (Kd: 19.5nM) to IL-2 (Fig. 4b-(3)). The number of the high-affinity IL-2R expressed Jαβ-2 cells and Jαβ-10 was comparable to that of express d IL-2Rβ molecules. In addition, treatment of these cells with Mik-β1 antibody completely abolished high-affinity IL-2 binding sites from the cell surface, while retaining the expression of low-affinity IL-2R (Fig. 4b-(4), (5)). These observations demonstrate unequivocally that the cDNA-encoded IL-2Rβ mol cule is directly involved in the formation of high-affinity r ceptor complex in association with the IL-2Rα chain. In contrast to the

aforedescribed T cell transformants, the Fβ-3 cells did not display any IL-2 binding on the cell surface under sam binding conditions. Interestingly the same observation was made with monkey COS cells that express the β chain, but failed to bind IL-2 (28). Thus, the results suggest the involvement of either a cell-type specific processing mechanism(s) or an additional cellular component(s), or both for the functional IL-2Rβ chain expression.

In order to characterize further the molecular structure of reconstituted IL-2R, we performed chemical crosslinking experiments with ^{125}I-IL-2 and non-cleavable chemical crosslinker, dissuccinimidyl suberate (DSS).

Fig. 5 illustrates the results of the affinity crosslinking studies of the IL-2R-positive transformants. Cells were incubated with 5nM (lanes 1-13) or 100pM (lanes 14-16) of ^{125}I-IL-2 in the absence (lanes 1-4, 14-16) or presence of a 250-fold molar excess of unlabeled IL-2 (lanes 5-7), 500-fold molar excess of affinity column-purified Mik-β1 (lanes 8-10) or 500-fold molar excess of affinity column-purified anti-Tac (lanes 11-13). Then cells were chemically crosslinked with dissuccinimidyl suberate (DSS) as described previously (16). The cells were then solubilized and the supernatants were subjected to 7.5% SDS-PAGE. Cells used were: Jurkat (lane 1); Jα-5 (lanes 2, 5, 8, 11, 14); Jβ-8 (lanes 3, 6, 9, 12, 15); Jαβ-10 (lanes 4, 7, 10, 13, 16). YT cells crosslinked with ^{125}I-IL-2 were used as a marker (M).

As can be seen cells expressing only IL-2Rβ chain were crosslinked with ^{125}I-labeled IL-2 and analysed by SDS-PAGE, a doublet band consisting of 90kD major and 85kD minor was detected and its migration profile was indistinguishable from that of YT cells (see arrows in Fig. 5 and ref. 16, 17). The appearance of the doublet is inhibited by an excess of unlabeled IL-2 or by Mik-β1. The doublet formation may be due to degradation of receptor-IL-2 complex. It is also possible that both protein products are derived by a differential post-translational modification(s). Alternatively, one of the doublet may represent a third component of the receptor complex. A broad band migrating around the position of 150kD was also detected in the transfectant (Jαβ-10) as well as YT cells. The appearance of this band is also inhibited by either unlabeled IL-2 or Mik-β1. It may represent the ternary complex of IL-2, IL-2Rα and Il-2Rβ molecules. In a series of chemical cross-linking experiments shown in Fig. 4, it was demonstrated that the physico-chemical properties of the receptor complex expressed on the surface of Jαβ-2 are indistinguishable from the properties of high-affinity receptor expressed on cultured T cells or PBLs (12, 16, 17).

Preliminary results of experiments to determine whether the expression of the α and β chains in non-lymphoid cells results in the formation of high-affinity receptor indicate that, when the α and β chain cDNAs are co-expressed transiently in COS cells, both chains can crosslink with ^{125}I-IL-2 at the concentration (400 pM) in which the similarly expressed α chain alone can not (28). The results may suggest the formation of the αβ heterodimeric receptor in this non-lymphoid cell line.

IL-2 internalization by reconstituted receptors

It has been reported that intermediate- and high-affinity IL-2 receptors can both internalize IL-2 (33-35). Ligand internalization is usually accompanied with the IL-2 signal transduction, suggesting this process to be essential.

Fig. 6 illustrates IL-2 internalization via the reconstituted receptors. IL-2 internalization was examined according to a method described previously (33). Briefly, cells ($5x10^7$) were treated with ^{125}I-IL-2 at a final concentration of 200pM (Jαβ-10) or 5nM (Jα-5, Jβ-8 and ELβ-13) at 0°C for 30 min. After washing, cells were suspended with prewarmed culture medium (37°C) and the kinetics of IL-2 internalization was examined as described previously (33). (a) ELβ-13, (b) Jβ-8, (c) Jαβ-10, (d) Jα-5.
(-●-●-●-), internalized IL-2; (...o...o...), cell-surface bound IL-2; (-■-■-■-■-), free IL-2.

As shown in Fig. 6, we examined whether the reconstituted receptors can internalize IL-2. In fact, the cells expressing IL-2Rβ chain alone, or both α and β chains are capable of internalizing IL-2 following a kinetic pattern similar to that reported for the native receptor. In contrast, the Jurkat cells expressing only IL-2Rα failed to internalize IL-2, similar to previously reported observations (33, 34). Preliminary results indicate that the growth of the cells expressing the intermediate- or high-affinity receptors is selectively inhibited by IL-2 (14, 36). W also have preliminary results that the β chain xpr ssed in another host cell line functions in stimulating the cell growth in response to IL-2 (28).

Cloning of Murine IL-2R receptor β chain

No specific antibodies to murin IL-2 receptor β-chain ar known to exist, accordingly the scr ening

method used for the isolation of cDNA for Hu IL-2Rβ chain was not employed.

A cDNA library was prepared using poly (A)$^+$-RNA from Concanavalin A stimulated mouse spleen cells; the cDNA was cloned in λgt 10 which was multiplied in E. coli.

Screening of this library was then carried out using the abov describ d human IL-2Rβ chain cDNA as the probe under non-stringent conditions. From the positive clones a clone designated λMIL 2Rβ-26 was selected. The cDNA insert in this clone contained only a 540 bp sequence of th whole murine Il-2Rβ chain sequence. This sequence was therefore isolated by digestion of λMIL-2Rβ-26 using Pvu 2 and used for screening another cDNA library prepared using poly (A)$^+$ from the mouse thymoma cell line EL-4 according to standard procedures and cloned into the BstXI site of the CDM8 vector followed by transfecting E. coli.

Screening of the cDNA library was carried out under highly stringent conditions according to the method described in European Patent Application No. 88 119 602.9 and Kashima et al. (Nature Vo. 313 pp 402-404, 1985).

From the positive clones clone pMIL-2Rβ-36 containing the structural gene for murine IL-2Rβ (see Fig. 8) was selected. The restriction map of the cDNA clone is shown in Fig. 7.

Plasmid pMIL-2Rβ-36 has been deposited in strain E. coli MC 1061/P3 on May 23, 1989 at the Fermentation Research Institute according the Budapest Treaty under accession number FERM BP-2435.

Preparation of soluble human interleukin 2 receptor β-chain

A secreted form of the hIL2-Rβ chain (termed hereafter soluble β) was produced by transfecting NIH 3T3 fibroblasts with the modified β-chain cDNA ("anchor minus" cDNA) lacking the entire DNA sequence encoding both intracytoplasmic and transmembrane domains of the native β-chain.

Construction of the expression vector harboring the anchor minus cDNA which encodes the soluble β - (BCMGNeo-sol.β)

The β-chain cDNA (Fig. 1b) was modified into the anchor minus form for the production of the soluble β. A strategy to generate the expression vector containing the anchor minus cDNA is illustrated in Fig. 9. First, the plasmid pIL-2Rβ30 containing the 2.3-kb β-chain cDNA in the CDM8 vector was digested with BssH II and Sma I (all restriction enzymes were purchased from New England BioLabs, Beverly, MA, USA), and a 1.9-kb cDNA fragment (base 58-1967) including the entire coding sequence (base 121-1773) of the β-chain was obtained. After fill-in at the BssH II end, the 1.9-kb cDNA was inserted into a Sma I restriction site of pBluescript SK vector (Stratagene, San Diego, CA, USA). This pBluescript SK-β1.9 plasmid was then digested with Sty I (restriction sites; base 825, 934 and 1235) and Sma I so that all of the intracytoplasmic and transmembrane regions were deleted, leaving bases 121-840 representing most of the extracellular region intact. Next, a 12-base synthetic linker (New England BioLabs, #1060) containing multiple termination codons (TAG) as well as the recognition sequence for Nhe I was phosphorylated and ligated to the Sty I/Sma I-digested plasmid DNA with T4 DNA ligase. After digestion with the Nhe I to remove excess linker, the DNA was ligated to the SK vector to construct pBluescript SK-sol.β.

This pBluescript SK-sol.β was digested with Sal I and Not I (the restriction sites were located within th polylinker region of the pBluescript SK vector), and the resulting 0.8-kb cDNA fragment encoding the soluble B was isolated. This cDNA fragment was introduced into Xho I/Not I-digested BCMGNeo vector (see Karasuyama et al., J. Exp. Med. 169: 13-25, 1989) containing the cytomegalovirus (CMV) promoter and neomycin-resistance gene to generate the final expression plasmid BCMGNeo-sol.β. The BCMGNeo is a shuttle vector containing 69 % of bovine papilloma virus (BPV) sequences which ensure extrachromosomal replication in mammalian cells. As illustrated in Fig. 10, which illustrates the nucleotide sequence and corresponding amino acid sequence for the native and soluble β, the soluble β cDNA encodes a mature protein consisting of 212 amino acids (aa) accompanied by a signal peptide of 26 aa, while the native β-chain cDNA encodes a membrane protein consisting of a signal peptide (26 aa), extracellular (214 aa), transmembrane (25 aa), and intracytoplasmic (286 aa) domains. The nucleotide and corresponding amino acid sequences for the native and soluble β are also illustrated in Fig. 10.

Transfection of NIH 3T3 fibroblast with BCMGNeo-sol.β and establishment of stable transformants secreting the soluble β

cDNA transfection was performed by the protoplast fusion technique as described in Karasuyama et al. (supra.). Briefly, bacteria, containing the BCMGNeo-sol.β were converted to protoplasts and fused with a murine fibroblast cell line, NIH 3T3 by using polyethylene glycol 2,000 (Wako Chemical Industri s, Osaka, Japan). Ten million protoplast-fused NIH 3T3 cells were then seeded in four 24-well plates. Twenty five days after the culture in RPMI 1640 medium containing 10 % fetal calf serum (FCS) and 750 µg/ml of G418 (Geneticin; Sigma, St. Louis, MO, USA), transformant-cell growth was observ d in 60 wells out of 104. When determined by the sandwich enzyme-linked immunosorbent assay (ELISA) as described below, culture supernatants from 18 wells out of 60 were found to be positive for the soluble β. Five clones were established by limiting dilution from a well which gave the highest absorbance in the ELISA, and they were all found to secret high levels of soluble β (Table I). In contrast, NIH 3T3 cells transfected with the full-length β-chain cDNA (designated 3T3-β11) did not secrete the β-chain molecule to any extent. In the subsequent studies, we used the clone designated 3T3-B4-14 which secreted the highest amount of the soluble β.

Table 1

Levels of the soluble β in the culture supernatant of NIH 3T3 fibroblats transfected with BCMGNeo-sol.β	
Culture supernatant	Absorbance at 405 nm in ELISA
3T3-B4-1	1.492
3T3-B4-4	1.301
3T3-B4-7	1.259
3T3-B4-14	1.579
3T3-B4-19	1.533
3T3-β11	0.052
medium alone	0.072

ELISA for detecting the soluble β

Culture supernatants of transfected cells were screened for the presence of the soluble β by a sandwich enzyme-linked immunosorbent assay (ELISA). In this assay were used two monoclonal antibodies Mik-β1 and -β3, supra. and Tsudo et al., Proc. Natl. Acad.Sci. USA, 86: 1982-1986, 1989, which recognize the distinct epitopes on the β-chain; i.e. Mik-β1 recognises the IL-2 binding site, while Mik-β3 recognises the epitope not involved in the IL-2 binding. As illustrated in Fig. 11, which is a schematic representation of the Sandwich ELISA Immulon-I microtiter plates (Dynatec, Chantilly, VA, USA) were coated overnight with 50 µl of Mik-β3 at 10 µg/ml in Tris-buffered saline (10 mM Tris-HCl, pH 7,4, 0,15 M NaCl). After discarding excess antibody, unbound sites were blocked by incubating with TBS containing 1 % bovine serum albumin for 1 hour. After washing with TBS containing 0,05 % Tween 20 (T-TBS), 50 µl of culture supernatants of the transformants were added to the wells and incubated for 1 hour. After washing, 50 µl of biotinylated Mik-β1 at 1 µg/ml were added as the secondary antibody to detect the soluble β bound to the primary antibody, Mik-β3 on the plate. After 45 minutes incubation and subsequent washing, 50 µl of alkaline phosphatase-conjugated avidin (Tago, Burlingame, CA, USA) were added. After a 45 minutes incubation, the plates were washed, 100 µl of p-nitrophenyl phosphate were added, and the absorbance of the wells was d termined at 405 nm after 45 minutes.

Apparent molecular weight of secreted soluble β

In order to define the molecular size of the soluble β, the 3T3-B4-14 cells were biosynthetically labeled with ^{35}S-methionine, and the soluble β immunoprecipitated by the Mik-β1 mAb from the culture super-

natant. Various amounts of immuno precipitates using the Mik-β1 and, as control UPC 10 mAb for precipitation were loaded and electrophoresed on an 8 % SDS-polyacrylamide gel. As shown in Fig. 12, when examined by the SDS-polyacrylamide gel electrophoresis (PAGE), the Mik-β1, but not the control UPC 10 mAb, identified a single species of protein with an apparent Mr of 37,000 in the culture supernatant of 3T3-B4-14 cells. This molecular size is in good agreement with that predicted for the truncated β-chain lacking all of the transmembrane (25 aa) and intracytoplasmic (286 aa) regions.

IL-2 binding ability of the soluble β

It was then investigated whether the secreted form of the β-chain is capable of binding IL-2. To this end, a "competitive" sandwich ELISA was employed. In this assay, the soluble β in the culture supernatant was fixed on the solid phase by the Mik-β3 mAb, a non-inhibitory mAb for IL-2 binding, so that the putative IL-2 binding site on the β-chain would remain unoccupied. Then, serial dilutions of IL-2 or unlabeled Mik-β1 were added as competitors for biotinylated Mik-β1. Fig. 13 shows the results of the competitive sandwich ELISA in which the curve -●-●- is for serial dilutions with unlabeled Mik-β1 and -o-o- for serial dilutions of IL-2. As shown in Fig. 13 unlabeled Mik-β1 reduced the absorbance dose-dependently, showing the specificity of this system. Likewise, IL-2 efficiently competed dose-dependently with biotinylated Mik-β1 for the binding to the soluble β, indicating that the soluble β is indeed capable of binding IL-2.

This competition curve is quite similar to that found for the detergent-solubilised native β-chain from YTS cells which express the β-chain alone, indicating that the affinity of the soluble β to IL-2 is comparable to that of the solubilised native β-chain.

The availability of the genes encoding IL-2Rβ chains makes it possible to explore novel approaches for the functional studies of the IL-2 system. The receptor structure operating in the IL-2 system is unique in that two structurally distinct membrane molecules, the IL-2Rα and IL-2Rβ chains, both bind IL-2 independently. The series of cDNA expression examples described herein substantiate further the previous notion that the α and β chains constitute the high-affinity IL-2R complex via a non-covalent association of the molecules (18, 37). Thus the peculiarity of this system is the involvement of three intermolecular interactions between one ligand and two distinct receptors. By virtue of the present invention it will now be possible to elucidate functional domains of this unique cytokine receptor system. Mutational analyses of the cloned β chain cDNA may provide clues as to the identification of respective domains involved in ligand binding and association with the α chain. To date, little is known about the cascade of biochemical events triggered by cytokines interacting with their homologous receptors. By the present invention we have demonstrated the presence in the IL-2Rβ chain of a large cytoplasmic region which most likely is involved in driving the IL-2 signal pathway(s). The particular acidic nuclei found in the cytoplasmic region may suggest coupling to other cytoplasmic signal transducers. Alternatively, in view of a previous report on the presence of IL-2 within the nucleous (33), an intriguing possibility is that the acidic as well as the proline-rich regions of the IL-2Rβ cytoplasmic component may play a role in activation of the genetic programming. The availability of the expression system in which the cDNA-encoded β chain can deliver growth signals will allow further clarification of the functional domaines of the receptor. It is now possible to study the essential role of IL-2 in the development and regulation of the immune system.

The availability of soluble counterparts to the cell surface receptor β-chain should facilitate structural analysis of the β-chain since crystallisation of soluble molecules is more easily accomplished than insoluble ones. The soluble molecules can also be used to neutralise the actual cell surface receptors for studies of the biological functions of the receptors or for therapeutic purposes.

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Claims

1. A recombinant DNA molecule coding for the β-chain of an IL-2 receptor or a portion thereof.
2. A recombinant DNA molecule as defined in claim 1 coding for the β-chain of human or murine IL-2 receptor or a portion thereof.
3. A recombinant DNA molecule characterized by a structural gene having the formula:

ATG
GCG GCC CCT GCT CTG TCC TGG CGT CTG CCC CTC CTC ATC
CTC CTC CTG CCC CTG GCT ACC TCT TGG GCA TCT GCA GCG
GTG AAT GGC ACT TCC CAG TTC AGA TGC TTC TAC AAC TCG
AGA GCC AAC ATC TCC TGT CTC TGG AGC CAA GAT GGG GCT
CTG CAG GAC ACT TCC TGC CAA GTC CAT GCC TGG CCG GAC
AGA CGG CGG TGG AAC CAA ACC TGT GAG CTG CTC CCC GTG
AGT CAA GCA TCC TGG GCC TGC AAC CTG ATC CTC GGA GCC
CCA GAT TCT CAG AAA CTG ACC ACA GTT GAC ATC GTC ACC
CTG AGG GTG CTG TGC CGT GAG GGG GTG CGA TGG AGG GTG
ATG GCC ATC CAG GAC TTC AAG CCC TTT GAG AAC CTT CGC
CTG ATG GCC CCC ATC TCC CTC CAA GTT GTC CAC GTG GAG
ACC CAC AGA TGG AAC ATA AGC TGG GAA ATC TCC CAA GCC
TCC CAC TAC TTT GAA AGA CAC CTG GAG TTC GAG GCC CGG
ACG CTG TCC CCA GGC CAC ACC TGG GAG GAG GCC CCC CTG
CTG ACT CTC AAG CAG AAG CAG GAA TGG ATC TGC CTG GAG
ACG CTC ACC CCA GAC ACC CAG TAT GAG TTT CAG GTG CGG
GTC AAG CCT CTG CAA GGC GAG TTC ACG ACC TGG AGC CCC
TGG AGC CAG CCC CTG GCC TTC AGG ACA AAG CCT GCA GCC
CTT GGG AAG GAC ACC ATT CCG TGG CTC GGC CAC CTC CTC
GTG GGC CTC AGC GGG GCT TTT GGC TTC ATC ATC TTA GTG
TAC TTG CTG ATC AAC TGC AGG AAC ACC GGG CCA TGG CTG
AAG AAG CTC CTG AAG TGT AAC ACC CCA GAC CCC TCG AAG
TTC TTT TCC CAG CTG AGC TCA GAG CAT GGA GGA GAC GTC

CAG AAG TGG CTC TCT TCG CCC TTC CCC TGA TCG TCC TTC
AGC CCT GGC GGC CTG GCA CCT GAG ATC TCG CCA CTA GAA
GTG CTG GAG AGG GAC AAG GTG ACG CAG CTG CTC CTG CAG
CAG GAC AAG GTG CCT GAG CCC GCA TCC TTA AGC AGC AAC
CAC TCG CTG ACC AGC TGC TTC ACC AAC CAG GGT TAC TTC
TTC TTC CAC CTC CCG GAT GCC TTG GAG ATA GAG GCC TGC
CAG GTG TAC TTT ACT TAC GAC CCC TAC TCA GAG GAA GAC
CCT GAT GAG GGT GTG GCC GGG GCA CCC ACA GGG TCT TCC
CCC CAA CCC CTG CAG CCT CTG TCA GGG GAG GAC GAC GCC
TAC TGC ACC TTC CCC TCC AGG GAT GAC CTG CTG CTC TTC
TCC CCC AGT CTC CTC GGT GGC CCC AGC CCC CCA AGC ACT
GCC CCT GGG GGC AGT GGG GCC GGT GAA GAG AGG ATG CCC
CCT TCT TTG CAA GAA AGA GTC CCC AGA GAC TGG GAC CCC
CAG CCC CTG GGG CCT CCC ACC CCA GGA GTC CCA GAC CTG
GTG GAT TTT CAG CCA CCC CCT GAG CTG GTG CTG CGA GAG
GCT GGG GAG GAG GTC CCT GAC GCT GGC CCC AGG GAG GGA
GTC AGT TTC CCC TGG TCC AGG CCT CCT GGG CAG GGG GAG
TTC AGG GCC CTT AAT GCT CGC CTG CCC CTG AAC ACT GAT
GCC TAC TTG TCC CTC CAA GAA CTC CAG GGT CAG GAC CCA
ATC CAC TTG GTG TAG

or a portion thereof or a degenerate variant thereof.

4. A recombinant DNA molecule according to claim 1 characterized by a structural gene having th formula

ATG

GCT ACC ATA GCT CTT CCC TGG AGC CTG TCC CTC TAC GTC TTC CTC CTG CTC CTG GCT ACA CCT TCG GCA TCT GCA

GCA GTG AAA AAC TGT TCC CAT CTT GAA TGC TTC TAC AAC TCA AGA GCC AAT GTC TCT TGC ATG TGG AGC CAT GAA

GAG GCT CTG AAT GTC ACA ACC TGC CAC GTC CAT GCC AAG TCG AAC CTG CGA CAC TGG AAC AAA ACC TGT GAG CTA

ACT CTT GTG AGG CAG GCA TCC TGG GCC TGC AAC CTG ATC CTC GGG TCG TTC CCA GAG TCC CAG TCA CTG ACC TCC

GTG GAC CTC CTT GAC ATA AAT GTG GTG TGC TGG GAA GAG AAG GGT TGG CGT AGG GTA AAG ACC TGC GAC TTC CAT

CCC TTT GAC AAC CTT CGC CTG GTG GCC CCT CAT TCC CTC CAA GTT CTG CAC ATT GAT ACC CAG AGA TGT AAC ATA

AGC TGG AAG GTC TCC CAG GTC TCT CAC TAC ATT GAA CCA TAC TTG GAA TTT GAG GCC CGT AGA CGT CTT CTG GGC

CAC AGC TGG GAG GAT GCA TCC GTA TTA AGC CTC AAG CAG AGA CAG ACG TGG CTC TTC TTG GAG ATC CTG ATC CCT

AGT ACC TCA TAT GAG GTC CAG GTG AGG GTC AAA GCT CAA CGA AAC AAT ACC GGG ACC TGG AGT CCC TGG AGC CAG

CCC CTG ACC TTT CGG ACA AGG CCA GCA GAT CCC ATG AAG GAC ATC CTC CCC ATG TCA TGG CTC AGA TAC CTT CTG

CTG GTC CTT GGT TGT TTT TCT GGC TTC TTC TCC TGC GTC TAC ATT TTG GTC AAG XXX CGC TAC CTT GGG CCA TGG

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CTG AAG ACA GTT CTC AAG TGC CAC ATC CCA GAT CCT TCT GAG TTC TTC TCC CAG CTG AGC TCC CAG CAT GGG GCA
GAC CTT CAG AAA TGG CTC TCC TCG CCT GTC CCC TTG TCC TTC TTC AGC CCC AGT GGC CCT GCC CCT GAG ATC TCT
CCG CTG GAA GTG CTC CAC GCA CAT TCC AAG GCC GTG CAG CTG CTC CTG TTA CAG AAG GAC TCT GCC CCT TTA CCC
TCG CCC AGC GGC CAC TCA CAG GCC AGC TGC TTC ACC AAC CAG GGC TAC TTC. TTC TTC CAT CTG CCC AAT GCC TTC
GAG ATC GAA TCC TGC CAG GTG TAC TTC ACC TAT GAC CCC TGT GTC GAA GAG GAG GTC GAG GAG CAT CGG TCA AGG
CTG CCC GAG GCA TCT CCC CAC CCA CCT CTG CTC CCT CTG GCT GGA GAA CAG CAT GAC TAC TCT GCC TTC CCG CCC
AGG GAT GAC CTG CTG CTC TTC TCC CCG AGC CTC AGC ACC CCC AAC ACT GCC TAT GGG CGC AGC AGA GCC CCT GAA
GAA AGA TCT CCA CTC TCC CTG CAT GAG GGA CTT CCC TCC CTA GCA TCC CGT GAC CTG ATG GGC TTA CAG CGC CCT
CTG GAG CGG ATG CCG GAA GGT GAT GGA GAG GGG CTG TCT GCC AAT AGC TCT GGG GAG CAG GCC ACT GTC CCA GAA
GGC AAC CTT CAT GGG CAA GAT CAG GAC AGA GGC CAG GGC CCC ATC CTG ACC CTG ACC ACC GAT GCC TAT CTG TCT
CTT CAA GAA CTA CAG GCC CAA GAT TCA GTC CAC CTA ATA TAG
                                    ***

XXX = GGC or TGC
```

or a portion thereof or a degenerate variant thereof.

5. A recombinant DNA molecule consisting of the DNA insert of one of the following plasmids

pIL-2Rβ6,
pIL-2Rβ9,
pIL-2Rβ19,
pIL-2Rβ30.

pMIL-2Rβ36,
which codes for IL-2Rβ or a portion th reof.

6. A recombinant DNA molecule characterised in that it codes for a soluble portion of the entire IL2-Rβ chain or variant th reof as defin d in any one of claims 1 to 5.

7. A r combinant DNA molecule as defined in claim 6, characterised in that it codes for the amino acids about 1 to about 210 of the human IL2-Rβ chain.

8. A recombinant DNA molecule as defined in claim 7 in which the terminal nucleotides are as follows
GCC CTT GCT AGC TAG
and which codes for a derivative of the water soluble human IL-2Rβ chain.

9. A recombinant DNA molecule capable of hybridising to the recombinant DNA as defined in any one of the preceding claims and which codes for a protein having the activity of a IL-2Rβ chain or a soluble portion thereof as defined in claim 6.

10. A recombinant DNA molecule as defined in any one of claims 1 to 9 which further comprises regulatory sequences operably linked to the structural gene for the IL-2β chain or portion thereof.

11. A recombinant DNA molecule as defined in claim 10 which is a plasmid.

12. The plasmid
pIL-2Rβ6,
pIL-2Rβ9,
pIL-2Rβ19,
pIL-2Rβ30 or
pMIL-2Rβ36.

13. A host cell which has been transformed by a recombinant DNA molecule as defined in any one of claims 10 to 12.

14. A host cell as defined in claim 13, which is a bacterial cell or a yeast cell or a mammalian cell.

15. A protein having the structure defined by the cDNA according to any one of claims 1 to 9.

16. A hybridoma, sub-clone or mutant thereof capable of secreting a monoclonal antibody having a specific affinity to a protein as defined in claim 15.

17. A monoclonal antibody having a specific affinity to a protein as defined in claim 15.

18. A method of producing a hybridoma as defined in claim 16 which comprises immunizing a non-human animal with a protein as defined in claim 15, removing spleen cells from the immunized animal and fusing the spleen cells with non-immunoglobulin secreting myloma cells, and selecting from the resulting hybridomas a cell line which produces a monoclonal antibody having the desired binding specificity and, if desired, subsequently sub-cloning said hybridoma.

19. Process for preparing a DNA as claimed in any of the claims 1 to 10 comprising digesting a suitable vector with one or more suitable restriction endonucleases and isolating the desired DNA.

20. A process for preparing a protein as claimed in claim 15 which comprises transforming a suitable host organism with an expression vector containing a coding sequence as claimed in any of the claims 1 to 9 for the desired polypeptide at an appropriate site for expression and isolating the desired protein from the resulting transformants.

21. A process as claimed in claim 20 wherein an expression vector is used as claimed in any of the claims 10 to 12.

22. A process for the preparation of a monoclonal antibody as claimed in claim 17 which comprises cultivating a cell as defined in claim 16 and isolating the nonoclonal antibody produced thereby.

23. A process for preparing a host organism as claimed in claim 13 or 14, wherein a vector as claimed in any one of claims 10 to 12 is transformed with a suitable host.

24. A process for preparing a vector as claimed in any one of claims 10 to 12, wherein a DNA-sequence as claimed in any one of claims 1 to 9 is inserted in a suitable vector.

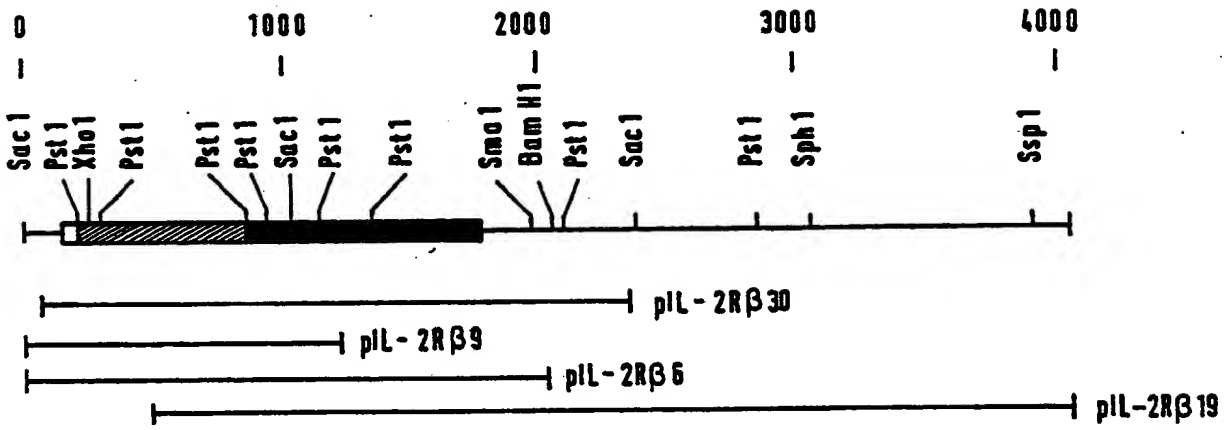

FIG. 1a

FIG. 1B

PAGE 1

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                                GCAGCCAGAGCTCAGCAGGGCCCTGGAGAGATGG      34
      CCACGGTCCCAGCACCGGGGAGGACTGGAGAGCGCGCGCTGCCACCGCCCC
      ATGTCTCAGCCAGGGCTTCCTTCCTCGGCTCCACCCTGTGGATGTA  ATG     134
                                                     Met
                                                     -26
      GCG GCC CCT GCT CTG TCC TGG CGT CTG CCC CTC CTC ATC     173
-25   Ala Ala Pro Ala Leu Ser Trp Arg Leu Pro Leu Leu Ile

      CTC CTC CTG CCC CTG GCT ACC TCT TGG GCA TCT GCA GCG     212
-12   Leu Leu Leu Pro Leu Ala Thr Ser Trp Ala Ser Ala Ala

      GTG AAT GGC ACT TCC CAG TTC AGA TGC TTC TAC AAC TCG     251
  2   Val Asn Gly Thr Ser Gln Phe Thr Cys Phe Tyr Asn Ser

      AGA GCC AAC ATC TCC TGT CTC TGG AGC CAA GAT GGG GCT     290
 15   Arg Ala Asn Ile Ser Cys Val Trp Ser Gln Asp Gly Ala

      CTG CAG GAC ACT TCC TGC CAA GTC CAT GCC TGG CCG GAC     329
 28   Leu Gln Asp Thr Ser Cys Gln Val His Ala Trp Pro Asp

      AGA CGG CGG TGG AAC CAA ACC TGT GAG CTG CTC CCC GTG     368
 41   Arg Arg Arg Trp Asn Gln Thr Cys Glu Leu Leu Pro Val

      AGT CAA GCA TCC TGG GCC TGC AAC CTG ATC CTC GGA GCC     407
 54   Ser Gln Ala Ser Trp Ala Cys Asn Leu Ile Leu Gly Ala

      CCA GAT TCT CAG AAA CTG ACC ACA GTT GAC ATC GTC ACC     446
 67   Pro Asp Ser Gln Lys Leu Thr Thr Val Asp Ile Val Thr

      CTG AGG GTG CTG TGC CGT GAG GGG GTG CGA TGG AGG GTG     485
 80   Leu Arg Val Leu Cys Arg Glu Gly Val Arg Trp Arg Val

      ATG GCC ATC CAG GAC TTC AAG CCC TTT GAG AAC CTT CGC     524
 93   Met Ala Ile Gln Asp Phe Lys Pro Phe Glu Asn Leu Arg

      CTG ATG GCC CCC ATC TCC CTC CAA GTT GTC CAC GTG GAG     563
106   Leu Met Ala Pro Ile Ser Leu Gln Val Val His Val Glu

      ACC CAC AGA TGG AAC ATA AGC TGG GAA ATC TCC CAA GCC     602
119   Thr His Arg Cys Asn Ile Ser Trp Glu Ile Ser Gln Ala

      TCC CAC TAC TTT GAA AGA CAC CTG GAG TTC GAG GCC CGG     641
132   Ser His Tyr Phe Glu Arg His Leu Glu Phe Glu Ala Arg

      ACG CTG TCC CCA GGC CAC ACC TGG GAG GAG GCC CCC CTG     680
145   Thr Leu Ser Pro Gly His Thr Trp Glu Glu Ala Pro Leu
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FIG.1B

PAGE 2

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       CTG ACT CTC AAG CAG AAG CAG GAA TGG ATC TGC CTG GAG     719
158    Leu Thr Leu Lys Gln Lys Gln Glu Trp Ile Cys Leu Glu

       ACG CTC ACC CCA GAC ACC CAG TAT GAG TTT CAG GTG CGG     758
171    Thr Leu Thr Pro Asp Thr Gln Tyr Glu Phe Gln Val Arg

       GTC AAG CCT CTG CAA GGC GAG TTC ACG ACC TGG AGC CCC     797
184    Val Lys Pro Leu Gln Gly Glu Phe Thr Thr Trp Ser Pro

       TGG AGC CAG CCC CTG GCC TTC AGG ACA AAG CCT GCA GCC     836
197    Trp Ser Gln Pro Leu Ala Phe Arg Thr Lys Pro Ala Ala

       CTT GGG AAG GAC ACC ATT CCG TGG CTC GGC CAC CTC CTC     875
210    Leu Gly Lys Asp Thr Ile Pro Trp Leu Gly His Leu Leu

       GTG GGC CTC AGC GGG GCT TTT GGC TTC ATC ATC TTA GTG     914
223    Val Gly Leu Ser Gly Ala Phe Gly Phe Ile Ile Leu Val

       TAC TTG CTG ATC AAC TGC AGG AAC ACC GGG CCA TGG CTG     953
236    Tyr Leu Leu Ile Asn Cys Arg Asn Thr Gly Pro Trp Leu

       AAG AAG CTC CTG AAG TGT AAC ACC CCA GAC CCC TCG AAG     992
249    Lys Lys Val Leu Lys Cys Asn Thr Pro Asp Pro Ser Lys

       TTC TTT TCC CAG CTG AGC TCA GAG CAT GGA GGA GAC GTC    1031
262    Phe Phe Ser Gln Leu Ser Ser Glu His Gly Gly Asp Val

       CAG AAG TGG CTC TCT TCG CCC TTC CCC TGA TCG TCC TTC    1070
275    Gln Lys Trp Leu Ser Ser Pro Phe Pro Ser Ser Ser Phe

       AGC CCT GGC GGC CTG GCA CCT GAG ATC TCG CCA CTA GAA    1109
288    Ser Pro Gly Gly Leu Ala Pro Glu Ile Ser Pro Leu Glu

       GTG CTG GAG AGG GAC AAG GTG ACG CAG CTG CTC CTG CAG    1148
301    Val Leu Glu Arg Asp Lys Val Thr Gln Leu Leu Leu Gln

       CAG GAC AAG GTG CCT GAG CCC GCA TCC TTA AGC AGC AAC    1187
314    Gln Asp Lys Val Pro Glu Pro Ala Ser Leu Ser Ser Asn

       CAC TCG CTG ACC AGC TGC TTC ACC AAC CAG GGT TAC TTC    1226
327    His Ser Leu Thr Ser Cys Phe Thr Asn Gln Gly Tyr Phe

       TTC TTC CAC CTC CCG GAT GCC TTG GAG ATA GAG GCC TGC    1265
340    Phe Phe His Leu Pro Asp Ala Leu Glu Ile Glu Ala Cys

       CAG GTG TAC TTT ACT TAC GAC CCC TAC TCA GAG GAA GAC    1304
353    Gln Val Tyr Phe Thr Tyr Asp Pro Tyr Ser Glu Glu Asp

       CCT GAT GAG GGT GTG GCC GGG GCA CCC ACA GGG TCT TCC    1343
366    Pro Asp Glu Gly Val Ala Gly Ala Pro Thr Gly Ser Ser

       CCC CAA CCC CTG CAG CCT CTG TCA GGG GAG GAC GAC GCC    1382
379    Pro Gln Pro Leu Gln Pro Leu Ser Gly Glu Asp Asp Ala
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FIG.1B

Neu eingereicht / Newly filed
Nouvellement déposé

PAGE 3

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      TAC TGC ACC TTC CCC TCC AGG GAT GAC CTG CTG CTC TTC    1421
392   Tyr Cys Thr Phe Pro Ser Arg Asp Asp Leu Leu Leu Phe

      TCC CCC AGT CTC CTC GGT GGC CCC AGC CCC CCA AGC ACT    1460
405   Ser Pro Ser Leu Leu Gly Gly Pro Ser Pro Pro Ser Thr

      GCC CCT GGG GGC AGT GGG GCC GGT GAA GAG AGG ATG CCC    1499
418   Ala Pro Gly Gly Ser Gly Ala Gly Glu Glu Arg Met Pro

      CCT TCT TTG CAA GAA AGA GTC CCC AGA GAC TGG GAC CCC    1538
431   Pro Ser Leu Gln Glu Arg Val Pro Arg Asp Trp Asp Pro

      CAG CCC CTG GGG CCT CCC ACC CCA GGA GTC CCA GAC CTG    1577
444   Gln Pro Leu Gly Pro Pro Thr Pro Gly Val Pro Asp Leu

      GTG GAT TTT CAG CCA CCC CCT GAG CTG GTG CTG CGA GAG    1616
457   Val Asp Phe Gln Pro Pro Pro Glu Leu Val Leu Arg Glu

      GCT GGG GAG GAG GTC CCT GAC GCT GGC CCC AGG GAG GGA    1655
470   Ala Gly Glu Glu Val Pro Asp Ala Gly Pro Arg Glu Gly

      GTC AGT TTC CCC TGG TCC AGG CCT CCT GGG CAG GGG GAG    1694
483   Val Ser Phe Pro Trp Ser Arg Pro Pro Gly Gln Gly Glu

      TTC AGG GCC CTT AAT GCT CGC CTG CCC CTG AAC ACT GAT    1733
496   Phe Arg Ala Leu Asn Ala Arg Leu Pro Leu Asn Thr Asp

      GCC TAC TTG TCC CTC CAA GAA CTC CAG GGT CAG GAC CCA    1772
509   Ala Tyr Leu Ser Leu Gln Glu Leu Gln Gly Gln Asp Pro

      ATC CAC TTG GTG TAG  ACAGATGGCCAGGGTGGGAGGCAGGCAGCT    1817
522   Thr His Leu Val ***

GCCTGCTCTGCGCCGAGCCTCAGAAGGACCCTGTTGAGGGTCCTCAGTCCA
CTGCTGAGGACACTCAGTGTCCAGTTGCAGCTGGACTTCTCCACCCGGATG
GCCCCCACCCAGTCCTGCACACTTGGTCCATCCATTTCCAAACCTCCACTG
CTGCTCCCGGGTCCTGCTGCCCGAGCCAGGAACTGTGTGTGTTGCAGGGGG
GCAGTAACTCCCCAACTCCCTCGTTAATCACAGGATCCCACGAATTTAGGC
TCAGAAGCATCGCTCCTCTCCAGCCCTGCAGCTATTCACCAATATCAGTCC
TCGCGGCTCTCCAGGGCTCCCTGCCCTGACCTCTTCCCTGGGTTTTCTGCC
CCAGCCTCCTCCTTCCCTCCCCTCCCCGTCCACAGGGCAGCCTGAGCGTGC
TTTCCAAAACCCAAATATGGCCACGCTCCCCCTCGGTTCAAAACCTTGCAC
AGGTCCCACTGCCCTCAGCCCCACTTCTCAGCCTGGTACTTGTACCTCCGG
TGTCGTGTGGGGACATCCCCTTCTGCAATCCTCCCTACCGTCCTCCCGAGC
CACTCAGAGCTCCCTCACACCCCCTCTGTTGCACATGCTATTCCCTGGGGC
TGCTGTGCGCTCCCCCTCATCTAGGTGACAAACTTCCCTGACTCTTCAAGT
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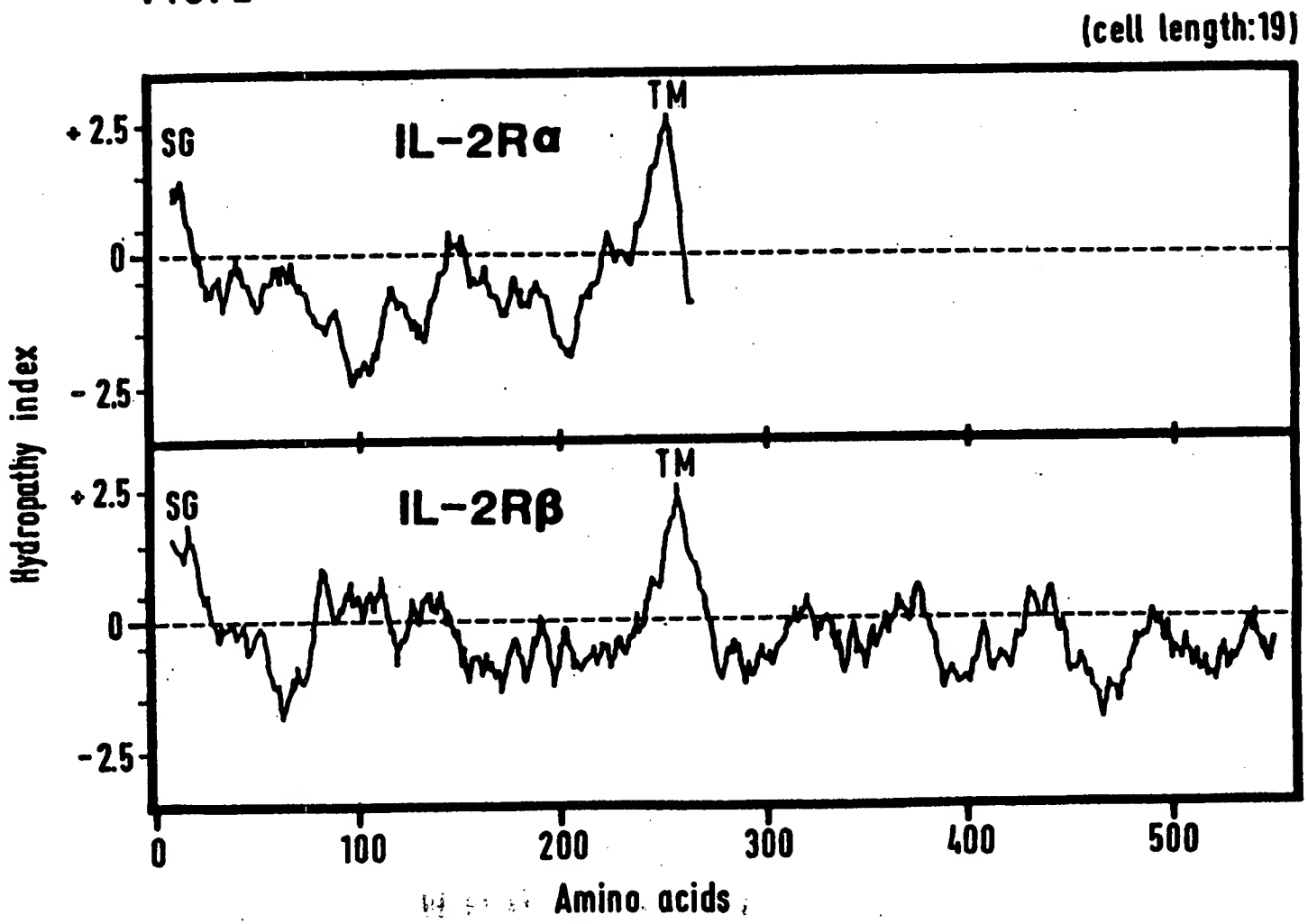
FIG. 1B

GCCGGTTTTGCTTCTCCTGGAGGGAAGCACTGCCTCCCTTAATCTGCCAGA
AACTTCTAGCGTCAGTGCTGGAGGGAGAAGCTGTCAGGGACCCAGGGCGCC
TGGAGAAAGAGGCCCTGTTACTATTCCTTTGGGATCTCTGAGGCCTCAGAG
TGCTTGGCTGCTGTATCTTTAATGCTGGGGCCCAAGTAAGGGCACAGATCC
CCCCGACAAAGTGGATGCCTGCTGCATCTTCCCACAGTGGCTTCACAGACC
CACAAGAGAAGCTGATGGGGAGTAAACCCTGGAGTCCGAGGCCCAGGCAGC
AGCCCCGCCTAGTGGTGGGCCCTGATGCTGCCAGGCCTGGGACCTCCCACT
GCCCCCTCCACTGGAGGGGTCTCCTCTGCAGCTCAGGGACTGGCACACTGG
CCTCCAGAAGGGCAGCTCCACAGGGCAGGGCCTCATTATTTTTCACTGCCC
CAGACACAGTGCCCAACACCCCGTCGTATACCCTGGATGAACGAATTAATT
ACCTGGCACCACCTCGTCTGGGCTCCCTGCGCCTGACATTCACACAGAGAG
GCAGAGTCCCGTGCCCATTAGGTCTGGCATGCCCCCTCCTGCAAGGGGCTC
AACCCCCTACCCCGACCCCTCCACGTATCTTTCCTAGGCAGATCACGTTGC
AATGGCTCAAACAACATTCCACCCCAGCAGGACAGTGACCCCAGTCCCAGC
TAACTCTGACCTGGGAGCCCTCAGGCACCTGCACTTACAGGCCTTGCTCAC
AGCTGATTGGGCACCTGACCACACGCCCCCACAGGCTCTGACCAGCAGCCT
ATGAGGGGGTTTGGCACCAAGCTCTGTCCAATCAGGTAGGCTGGGCCTGAA
CTAGCCAATCAGATCAACTCTGTCTTGGGCGTTTGAACTCAGGGAGGGAGG
CCCTTGGGAGCAGGTGCTTGTGGACAAGGCTCCACAAGCGTTGAGCCTTGG
AAAGGTAGACAAGCGTTGAGCCACTAAGCAGAGGACCTTGGGTTCCCAATA
CAAAAATACCTACTGCTGAGAGGGCTGCTGACCATTTGGTCAGGATTCCTG
TTGCCTTTATATCCAAAATAAACTCCCCTTTCTTGAGGTTGTCTGAGTCTT
GGGTCTATGCCTTGAAAAAAGCTGAATTATTGGACAGTCTCACCTCCTGCC
ATAGGGTCCTGAATGTTTCAGACCACAAGGGGCTCCACACCTTTGCTGTGT
GTTCTGGGGCAACCTACTAATCCTCTCTGCAAGTCGGTCTCCTTATCCCCC
CAAATGGAAATTGTATTTGCCTTCTCCACTTTGGGAGGCTCCCACTTCTTG
GGAGGGTTACATTTTTTAAGTCTTAATCATTTGTGACATATGTATCTATAC
ATCCGTATCTTTTAATGATCCGTGTGTACCATCTTTGTGATTATTTCCTTA
ATATTTTTTCTTTAAGTCAGTTCATTTTCGTTGAAATACATTTATAAAGAA
AAATCTTTGTTACTCTGTAAATGAAAAAACCCATTTTCGCTATAAATAAAA
GGTAACTGTACAAAATAAGTACAAT

FIG. 2



Nouvellement déposé

FIG. 3a

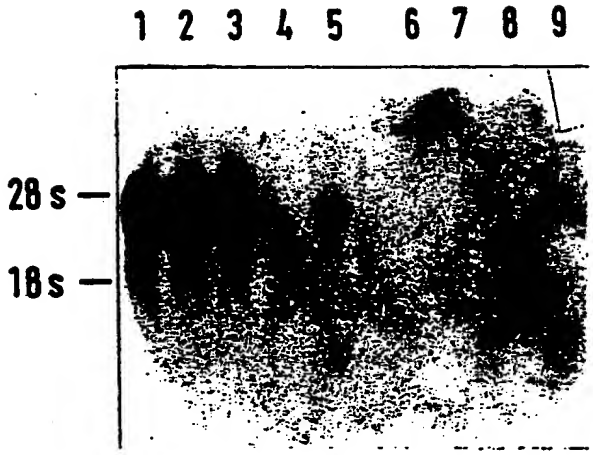


FIG. 3b

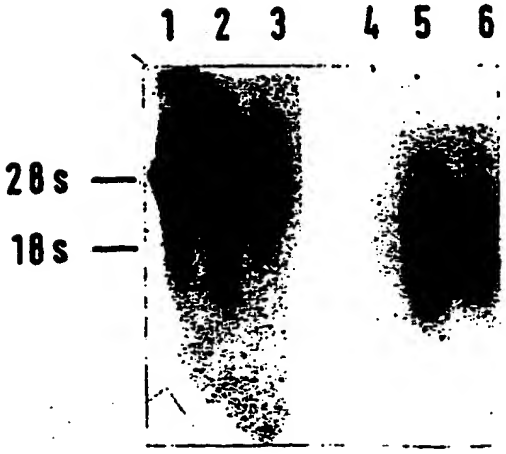
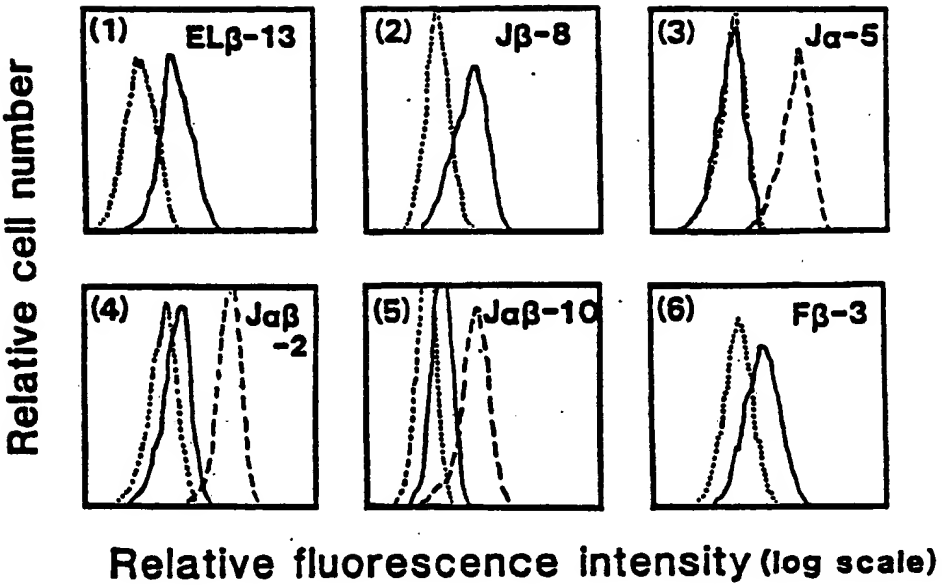


FIG.4a



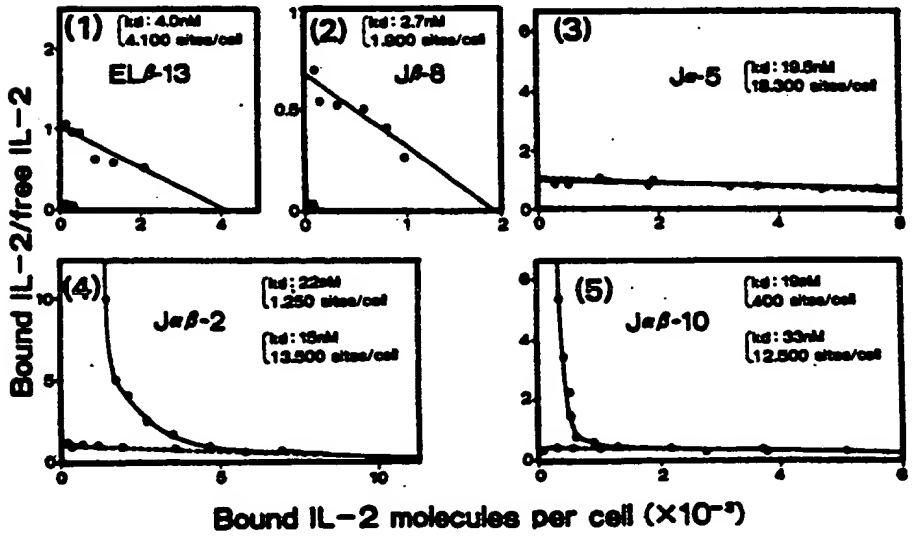
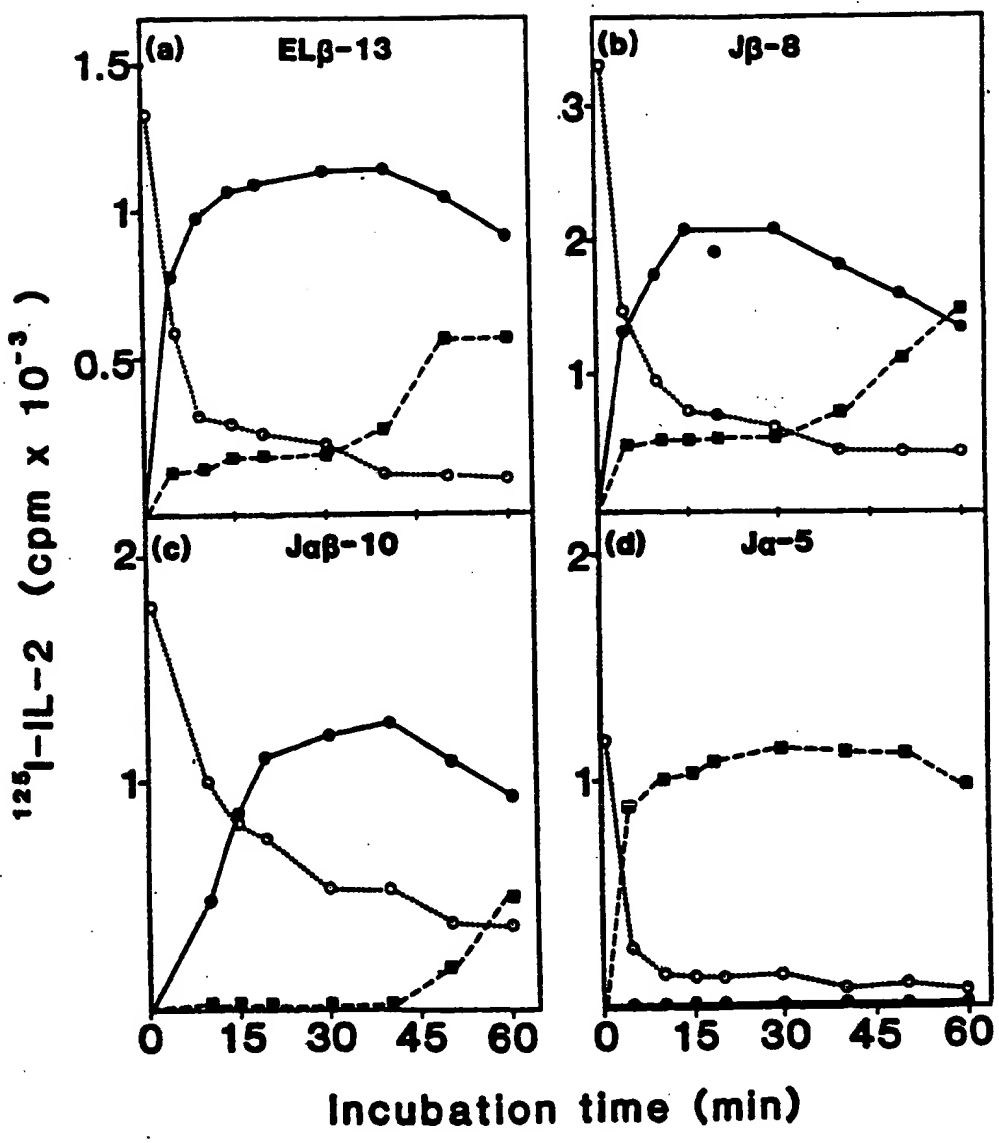


FIG.4b

FIG. 6



Restriction enzyme cleavage map of the mouse IL-2 receptor β chain cDNA clone

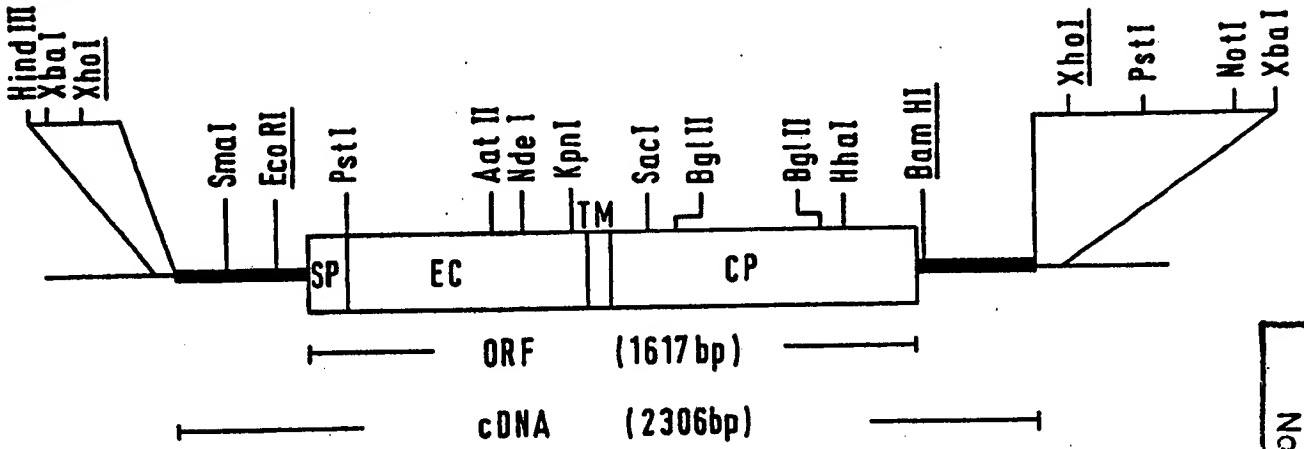


FIG. 7

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                    CGTTTTCTCTCTCTCTTGCTTCTTGCTCTCTTGCTTCTTACACGCTTGCTCCTGAAGATGTAAGAAATAAAGCTTTGCCGCA   82
GAAGATTCTGGTCTGTGGTGTTCTTCCTGGCCGGTCGTGAGAACGCGTCTAATAACAATTGGTGCCGAAACCCGGGACGAGAAAAAACTCGGGACGAGA   181
GTTTACATCACCGGTGCAAGGAAGATCCCTCATTCCAGAACCAGAACTGCGGGTCGCGGTAATAAAGGTTCCCGTAAAGCAGACTGTTAAGAAGGATTC   280
AACTGTATGAATTCAGAACTTTTCAGCTGGGGAACGAGAGATCCAGTGAGTACAGCTTTACGAGGGTTTGCATCCTCAGCTCCTCTCAGCTGTG  ATG   377
                                                                                                  Met

GCT ACC ATA GCT CTT CCC TGG AGC CTG TCC CTC TAC GTC TTC CTC CTG CTC CTG GCT ACA CCT TGG GCA TCT GCA   452
Ala Thr Ile Ala Leu Pro Trp Ser Leu Ser Leu Tyr Val Phe Leu Leu Leu Leu Ala Thr Pro Trp Ala Ser Ala

GCA GTG AAA AAC TGT TCC CAT CTT GAA TGC TTC TAC AAC TCA AGA GCC AAT GTC TCT TGC ATG TGG AGC CAT GAA   527
Ala Val Lys Asn Cys Ser His Leu Glu Cys Phe Tyr Asn Ser Arg Ala Asn Val Ser Cys Met Trp Ser His Glu

GAG GCT CTG AAT GTC ACA ACC TGC CAC GTC CAT GCC AAG TCG AAC CTG CGA CAC TGG AAC AAA ACC TGT GAG CTA   602
Glu Ala Leu Asn Val Thr Thr Cys His Val His Ala Lys Ser Asn Leu Arg His Trp Asn Lys Thr Cys Glu Leu

ACT CTT GTG AGG CAG GCA TCC TGG GCC TGC AAC CTG ATC CTC GGG TCG TTC CCA GAG TCC CAG TCA CTG ACC TCC   677
Thr Leu Val Arg Gln Ala Ser Trp Ala Cys Asn Leu Ile Leu Gly Ser Phe Pro Glu Ser Gln Ser Leu Thr Ser

GTG GAC CTC CTT GAC ATA AAT GTG GTG TGC TGG GAA GAG AAG GGT TGG CGT AGG GTA AAG ACC TGC GAC TTC CAT   752
Val Asp Leu Leu Asp Ile Asn Val Val Cys Trp Glu Glu Lys Gly Trp Arg Arg Val Lys Thr Cys Asp Phe His

CCC TTT GAC AAC CTT CGC CTG GTG GCC CCT CAT TCC CTC CAA GTT CTG CAC ATT GAT ACC CAG AGA TGT AAC ATA   827
Pro Phe Asp Asn Leu Arg Leu Val Ala Pro His Ser Leu Gln Val Leu His Ile Asp Thr Gln Arg Cys Asn Ile

AGC TGG AAG GTC TCC CAG GTC TCT CAC TAC ATT GAA CCA TAC TTG GAA TTT GAG GCC CGT AGA CGT CTT CTG GGC   902
Ser Trp Lys Val Ser Gln Val Ser His Tyr Ile Glu Pro Tyr Leu Glu Phe Glu Ala Arg Arg Arg Leu Leu Gly

CAC AGC TGG GAG GAT GCA TCC GTA TTA AGC CTC AAG CAG AGA CAG ACG TGG CTC TTC TTG GAG ATG CTG ATC CCT   977
His Ser Trp Glu Asp Ala Ser Val Leu Ser Leu Lys Gln Arg Gln Gln Trp Leu Phe Leu Glu Met Leu Ile Pro

AGT ACC TCA TAT GAG GTC CAG GTG AGG GTC AAA GCT CAA CGA AAC AAT ACC GGG ACC TGG AGT CCC TGG AGC CAG   1052
Ser Thr Ser Tyr Glu Val Gln Val Arg Val Lys Ala Gln Arg Asn Asn Thr Gly Thr Trp Ser Pro Trp Ser Gln

CCC CTG ACC TTT OGG ACA AGG CCA GCA GAT CCC ATG AAG GAG ATC CTC CCC ATG TCA TGG CTC AGA TAC CTT CTG   1127
Pro Leu Thr Phe Arg Thr Arg Pro Ala Asp Pro Met Lys Glu Ile Leu Pro Met Ser Trp Leu Arg Tyr Leu Leu

CTG GTC CTT GGT TGT TTT TCT GGC TTC TTC TCC TGC GTC TAC ATT TTG GTC AAG XXX CGG TAC CTT GGG CCA TGG   1202
Leu Val Leu Gly Cas Phe Ser Gly Phe Phe Ser Cys Val Tyr Ile Leu Val Lys  Y  Arg Tyr Leu Gly Pro Trp
```

FIG. 8

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251  CTG AAG ACA GTT CTC AAG TGC CAC ATC CCA GAT CCT TCT GAG TTC TTC TCC CAG CTG AGC TCC CAG CAT GGG GGA   1277
     Leu Lys Thr Val Leu Lys Cys His Ile Pro Asp Pro Ser Glu Phe Phe Ser Gln Leu Ser Ser Gln His Gly Gly

276  GAC CTT CAG AAA TGG CTC TCC TCG CCT GTC CCC TTG TCC TTC TTC AGC CCC AGT GGC CCT GCC CCT GAG ATC TCT   1352
     Asp Leu Gln Lys Trp Leu Ser Ser Pro Val Pro Leu Ser Phe Phe Ser Pro Ser Gly Pro Ala Pro Glu Ile Ser

301  CCG CTG GAA GTG CTC GAC GGA GAT TCC AAG GCC GTG CAG CTG CTC CTG TTA CAG AAG GAC TCT GCC CCT TTA CCC   1427
     Pro Leu Glu Val Leu Asp Gly Asp Ser Lys Ala Val Gln Leu Leu Leu Leu Gln Lys Asp Ser Ala Pro Leu Pro

326  TCG CCC AGC GGC CAC TCA CAG GCC AGC TGC TTC ACC AAC CAG GGC TAC TTC TTC TTC CAT CTG CCC AAT GCC TTG   1502
     Ser Pro Ser Gly His Ser Gln Ala Ser Cys Phe Thr Asn Gln Gly Tyr Phe Phe Phe His Leu Pro Asn Ala Leu

351  GAG ATC GAA TCC TGC CAG GTG TAC TTC ACC TAT GAC CCC TGT GTG GAA GAG GAG GTG GAG GAG GAT GGG TCA AGG   1577
     Glu Ile Glu Ser Cys Gln Val Tyr Phe Thr Tyr Asp Pro Cys Val Glu Glu Glu Val Glu Glu Asp Gly Ser Arg

376  CTG CCC GAG GGA TCT CCC CAC CCA CCT CTG CTG CCT CTG GCT GGA GAA CAG GAT GAC TAC TGT GCC TTC CCG CCC   1652
     Leu Pro Glu Gly Ser Pro His Pro Pro Leu Leu Pro Leu Ala Gly Glu Gln Asp Asp Tyr Cys Ala Phe Pro Pro

401  AGG GAT GAC CTG CTG CTC TTC TCC CCG AGC CTC AGC ACC CCC AAC ACT GCC TAT GGG GGC AGC AGA GCC CCT GAA   1727
     Arg Asp Asp Leu Leu Leu Phe Ser Pro Ser Leu Ser Thr Pro Asn Thr Ala Tyr Gly Gly Ser Arg Ala Pro Glu

426  GAA AGA TCT CCA CTC TCC CTG CAT GAG GGA CTT CCC TCC CTA GCA TCC CGT GAC CTG ATG GGC TTA CAG CGC CCT   1802
     Glu Arg Ser Pro Leu Ser Leu His Glu Gly Leu Pro Ser Leu Ala Ser Arg Asp Leu Met Gly Leu Gln Arg Pro

451  CTG GAG CGG ATG CCG GAA GGT GAT GGA GAG GGG CTG TCT GCC AAT AGC TCT GGG GAG CAG GCC AGT GTC CCA GAA   1877
     Leu Glu Arg Met Pro Glu Gly Asp Gly Glu Gly Leu Ser Ala Asn Ser Ser Gly Glu Gln Ala Ser Val Pro Glu

476  GGC AAC CTT CAT GGG CAA GAT CAG GAC AGA GGC CAG GGC CCC ATC CTG ACC CTG AAC ACC GAT GCC TAT CTG TCT   1952
     Gly Asn Leu His Gly Gln Asp Gln Asp Arg Gly Gln Gly Pro Ile Leu Thr Leu Asn Thr Asp Ala Tyr Leu Ser

501  CTT CAA GAA CTA CAG GCC CAA GAT TCA GTC CAC CTA ATA TAG  CAGGTGGCCAGGACTGGGATCCAGCTGCCTGGATCAGGTCAG   2036
     Leu Gln Glu Leu Gln Ala Gln Asp Ser Val His Leu Ile ***

     GCTTGAGGAAGACTGCTTAAGAGGTCTCTTGAGGACAGTCCACTGCTGAGGACTGTCCTGGCTATCCCTGCCCCCCCCCTCCAAACTTAATCATCCACT   2135
     TCTGAACTCCATTTGCTACTTCCTGGTCTAACCAGGGTTTGGTGGAGGGTGGGGAGTGGGGGAGCGGTGGTCAGCTCCACTGCCCTATTTAGTCATGAG   2234
     GTCACTAGCTTCCTGTACCTGCCTCCTTCCCTCCTGCTTGCCTTCACAGGGCAGCTAGAACTTGCTTCCCCG                               2306
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XXX = GGC or TGC

Y = Gly or Cys

FIG. 8

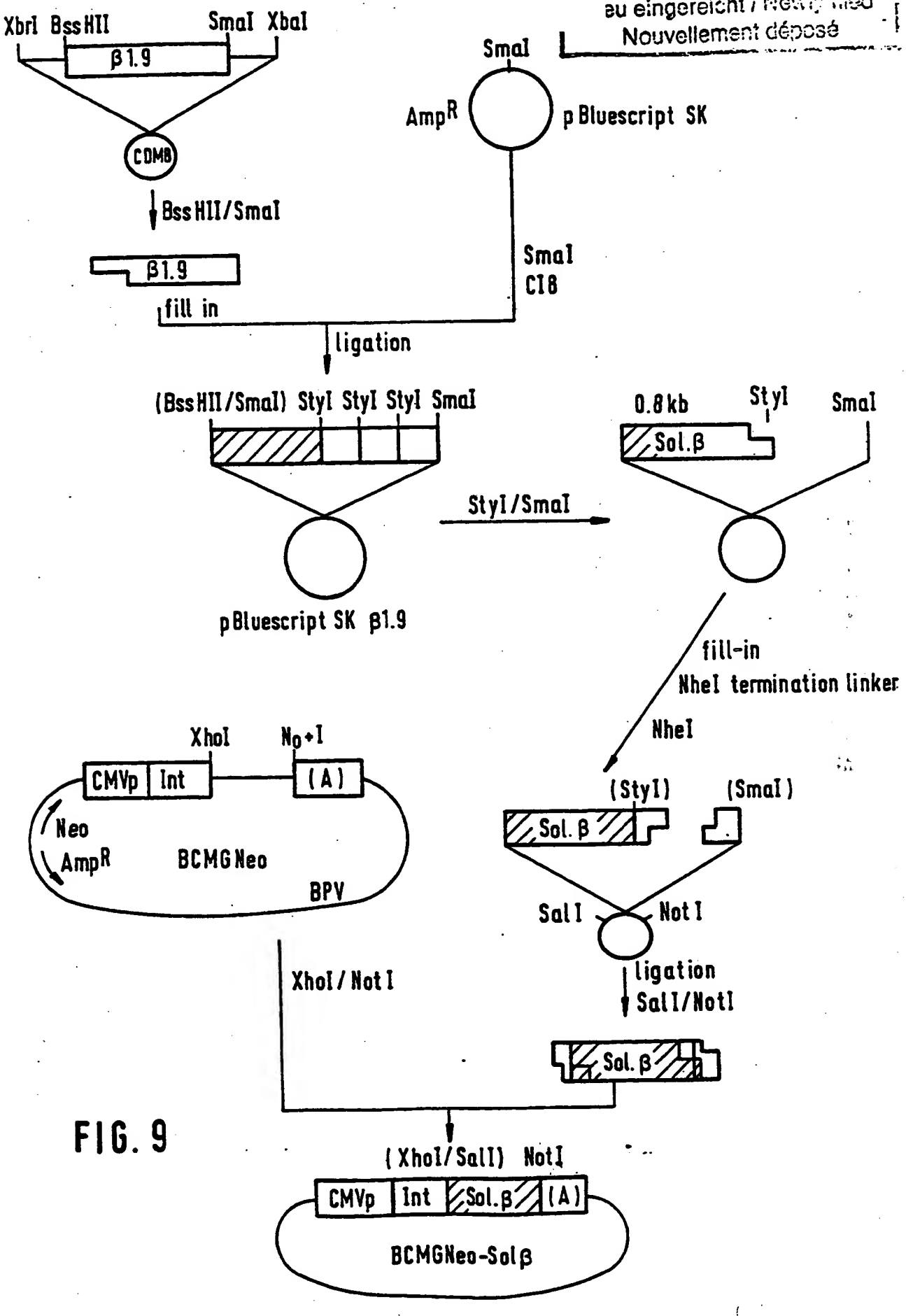
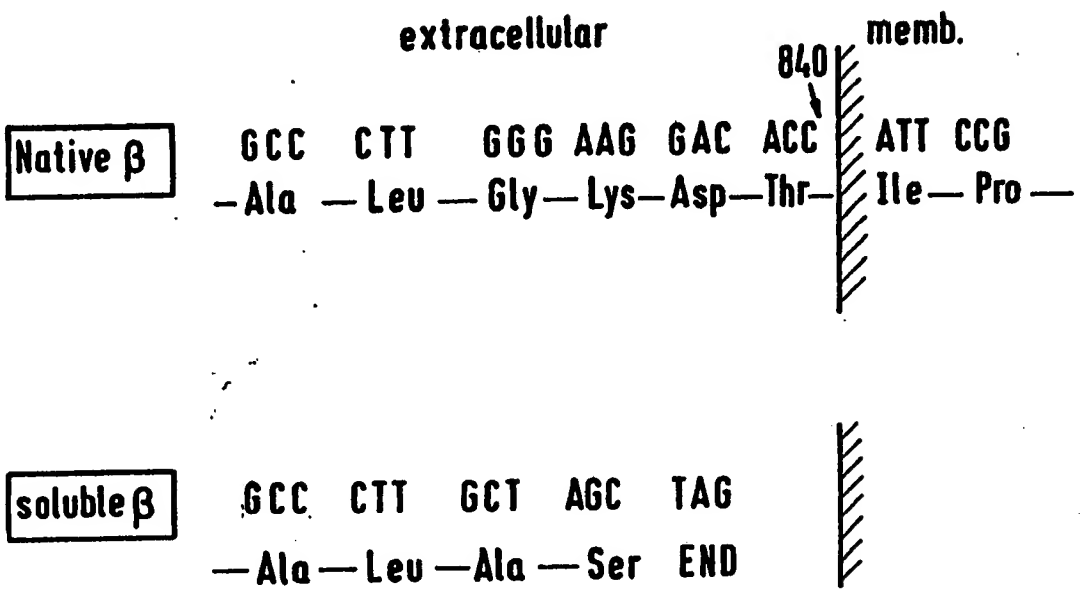
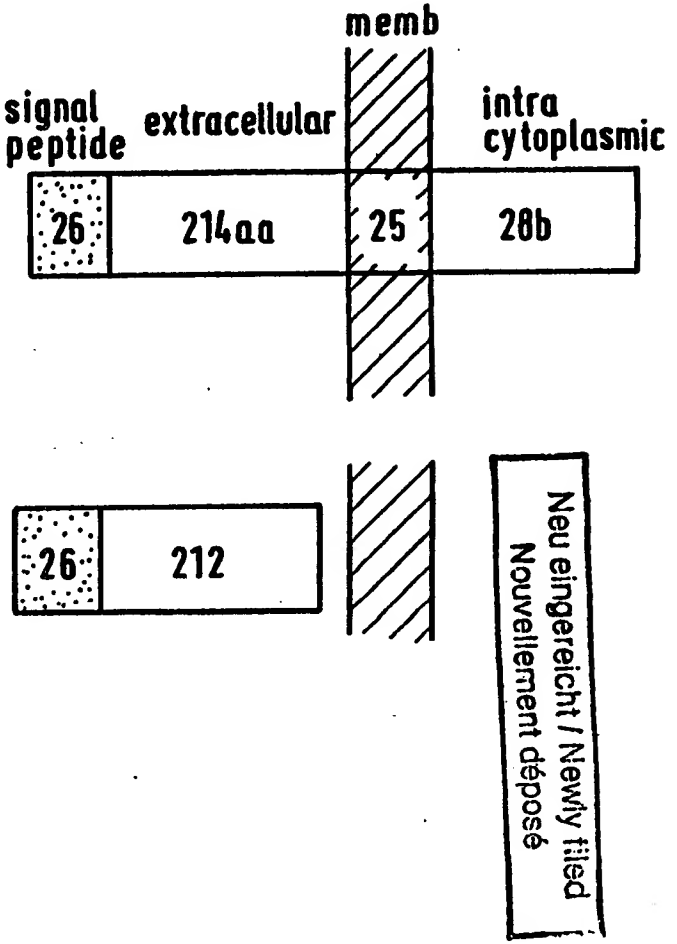


FIG. 9

FIG. 10


extracellular
memb.
840
Native β
GCC CTT GGG AAG GAC ACC ATT CCG
—Ala —Leu —Gly—Lys—Asp—Thr— Ile— Pro —
soluble β
GCC CTT GCT AGC TAG
—Ala—Leu—Ala—Ser END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signal peptide
extracellular
intra cytoplasmic
26
214aa
25
28b
26
212
Neu eingereicht / Newly filed
Nouvellement déposé

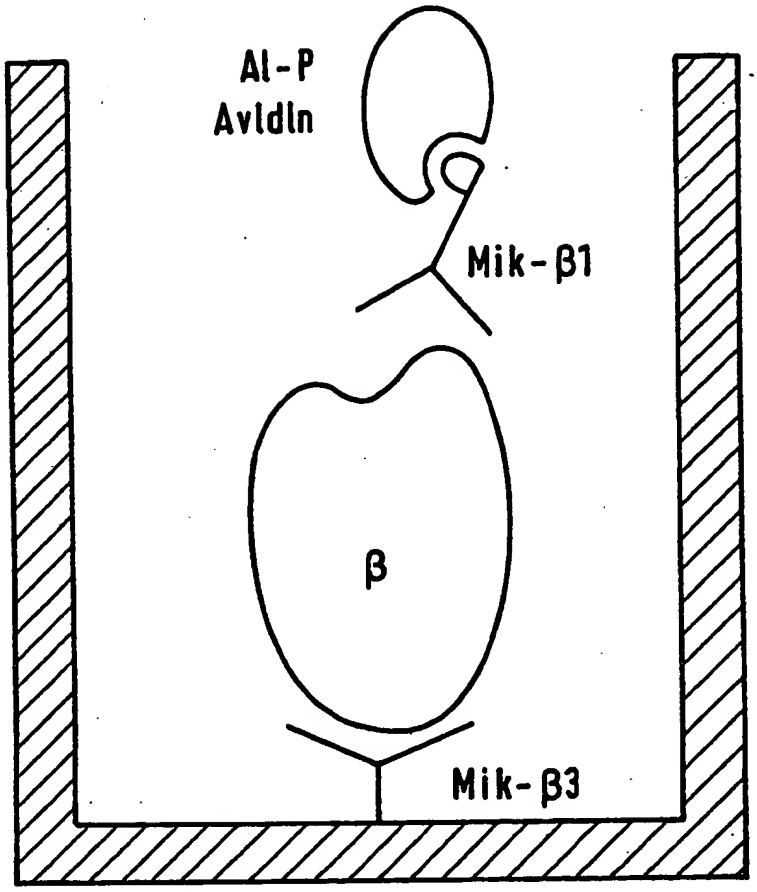


FIG. 11

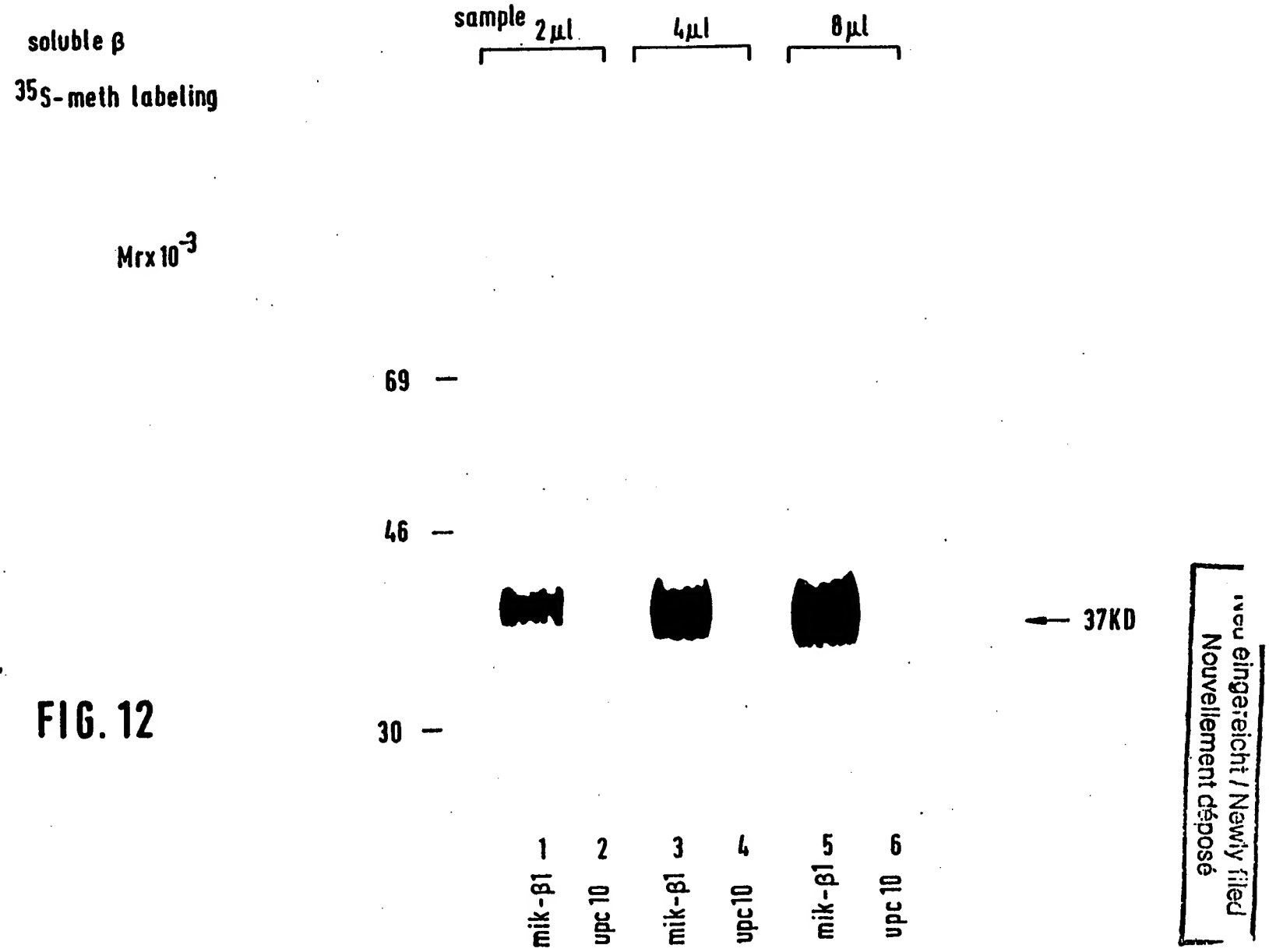
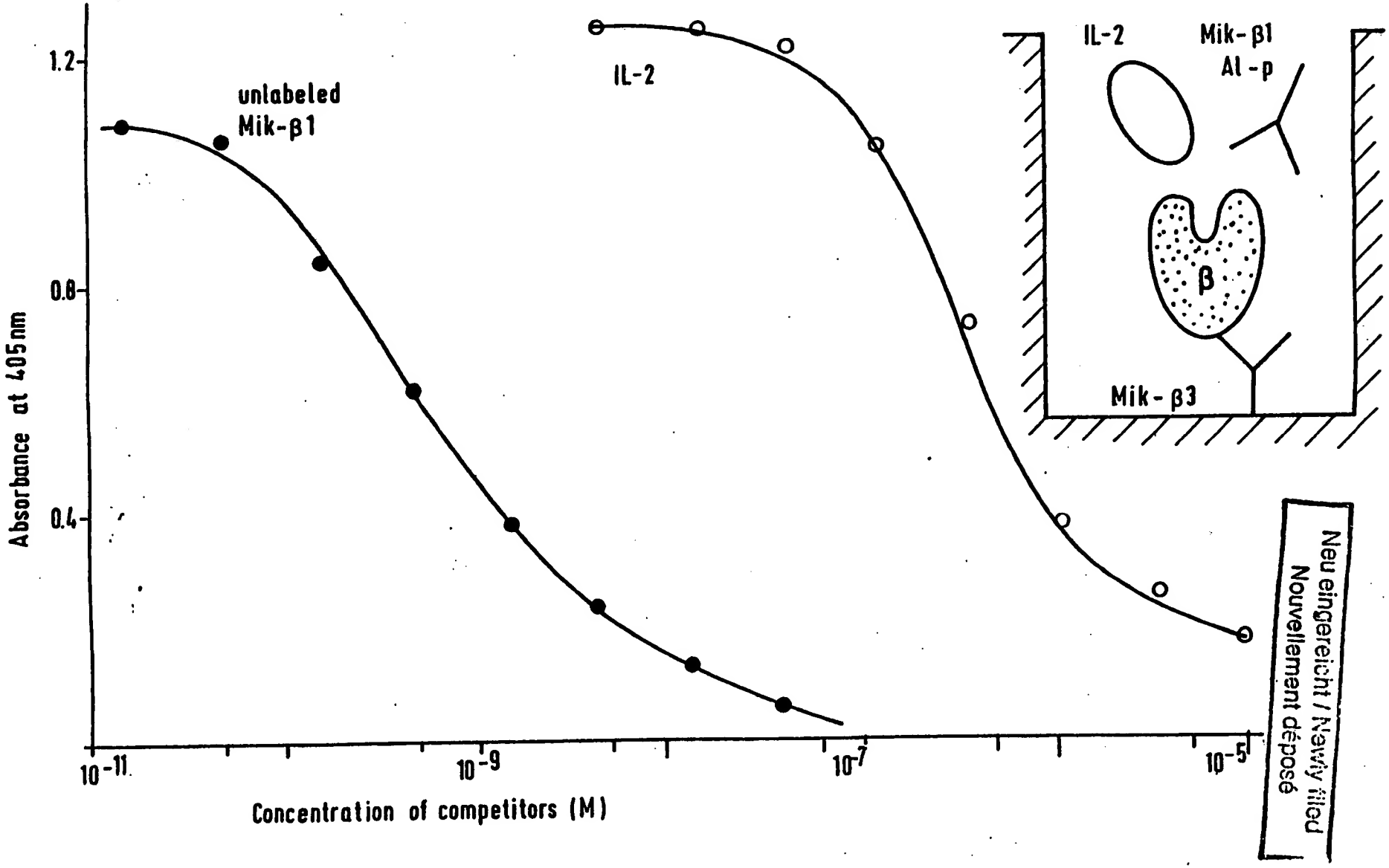


FIG. 12

FIG. 13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0.8
0.4
Absorbance at 405nm
unlabeled
Mik-β1
IL-2
10-11
10-9
10-7
10-5
Concentration of competitors (M)
IL-2
Mik-β1
Al-p
β
Mik-β3
Neu eingereicht / Newly filed
Nouvellement déposé

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EUROPEAN SEARCH REPORT

Application Number

EP 90 10 4246

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X Y	WO-A-8 900 168 (THE UNITED STATES OF AMERICA) * Page 6, line 1 - page 8, line 30 *	15-18, 22 1-14,19 -21,23, 24	C 12 N 15/12 C 12 P 21/08 C 12 P 21/02
Y	EP-A-0 162 699 (IMMUNEX CORP.)	1-14,19 -21,23, 24	
P,X	SCIENCE, vol. 244, 5th May 1989, pages 551-556, Washington, DC, US; M. HATAKEYAMA et al.: "Interleukin-2 receptor beta chain gene: Generation of three receptor forms by cloned human alpha and beta chain cDNA's" * Whole document *	1-24	
P,X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE, USA, vol. 86, March 1989, pages 1982-1986, Washington, DC, US; M. TSUDO et al.: "Characterization of the interleukin 2 receptor beta chain using three distinct monoclonal antibodies" * Page 1982: "Materials and methods" *	22	TECHNICAL FIELDS SEARCHED (Int. Cl.5) C 12 N C 12 P
P,X	THE JOURNAL OF IMMUNOLOGY, vol. 143, no. 12, 15th December 1989, pages 4039-4043, The American Association of Immunologists, US; M. TSUDO et al.: "Reconstitution of a functional IL-2 receptor by the beta-chain cDNA, a newly acquired receptor transduced negative signal"	6	

-/-

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	17-06-1990	VAN PUTTEN A.J.

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European Patent Office

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Application Number

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	THE JOURNAL OF IMMUNOLOGY, vol. 136, no. 11, 1st June 1986, pages 4099-4105, The American Association of Immunologists, US; B.F. TREIGER et al.: "A secreted form of the human interleukin 2 receptor encoded by an "Anchor minus" cDNA" ---	1-24	
X	IMMUNOBIOLOGY, vol. 175, no. 4, 1987, pages 343-344, Stuttgart, DE; G. KRUPPA et al.: "Transfer and expression of the human IL 2 receptor beta-chain gene in murine fibroblasts and T lymphoid cells using retroviral vectors" * Abstract * ---	1	
A	NATURE, vol. 327, no. 6122, 11th-17th June 1987, pages 518-522, Basingstoke, Hampshire, GB; M. DUKOVICH et al.: "A second human interleukin-2 binding protein that may be a component of high-affinity interleukin-2 receptors" -----		TECHNICAL FIELDS SEARCHED (Int. Cl.5)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	17-06-1990	VAN PUTTEN A.J.

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